Irreversible Enzyme Inhibitors. CII. On the Mode of Phenyl Binding of 9-Phenylguanine to Guanine Deaminase and Xanthine Oxidase[1-4]

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9-Phenylguanine inhibits guanine deaminase and xanthine oxidase 28 and 140 times, respectively, more effectively than 9-methylguanine due to a binding interaction of the phenyl group with the enzyme. The extent of phenyl binding to the enzymes was not influenced by electronegative or electropositive substituents; therefore any charge-transfer complex between the phenyl and the enzymes was rendered unlikely. In contrast, the binding of 9-phenylguanine to both enzymes was strongly influenced by polar or nonpolar substituents. Enhanced binding to guanine deaminase occurred with nonpolar substituents at either the *meta* or *para* position of the benzene ring, but enhanced binding to xanthine oxidase was observed only with *meta* substituents. The best inhibitor of guanine deaminase in this study was 9-(*p*-ethoxyphenyl)guanine which was complexed about 130 times more effectively than the substrate, guanine. The best inhibitors of xanthine oxidase in this study were 9-phenylguanines substituted in the *para* position with ethoxy or carboxy; these were complexed about 70 times more effectively than the substrate, hypoxanthine.

9-Phenylguanine (**2**) (Table I) is an excellent inhibitor of both guanine deaminase[4] and xanthine oxidase.[5] Comparison of 9-alkyl-, 9-benzyl-, and 9-phenylguanines showed that the in-plane phenyl group gave maximum hydrocarbon interaction with the two enzymes. The extent of this interaction can be gleaned by comparing 9-methylguanine (**1**) and 9-phenylguanine (**2**) as inhibitors of the enzymes. The 9-phenyl substituent of **2** gave 140-fold more effective binding to xanthine oxidase than the 9-methyl substituent of **1**[5] (Table I); similarly, **2** was a 28-fold more effective inhibitor of guanine deaminase than **1**.[4] Since the 9-phenyl group gave optimum hydrocarbon interaction with both enzymes the following questions were posed:[4-6] (a) can any charge-transfer character of the phenyl binding be observed by studying Hammett σ effects; and (b) does the phenyl group complex to the enzymes by hydrophobic binding and the accompanying van der Waal's forces, which can be shown by the effect of polar and nonpolar substituents?

The results of such studies could then be used to find better reversible inhibitors and to suggest where a leaving group should be placed on the phenyl substituent in order to obtain active-site-directed irreversible inhibitors of the two enzymes;[7,8] these studies on the mode of phenyl binding to xanthine oxidase and guanine deaminase are the subjects of this paper.

The inhibition studies of guanine deaminase[9] and xanthine oxidase[10] by 21 9-substituted guanines are listed in Table I. A phenyl group can complex to an enzyme in a donor–acceptor complex or by hydrophobic bonding with the accompanying van der Waal's forces or both.[6] If the benzene ring is binding in a donor–acceptor complex, then the extent of binding should be influenced by electron-donating or electron-withdrawing groups (Hammett σ effects) placed on the benzene ring. Whether these groups have a beneficial or detrimental effect is dependent upon whether the benzene ring is the donor or the acceptor partner in the donor–acceptor complex.

Guanine Deaminase.—That there was no correlation between binding and the σ value of the substituents is readily seen with the compounds in Table I. Note that 4 substituents on the benzene ring such as the electron-donating amino group of **10**, and the dimethylamino group of **9**, gave a twofold loss in binding, but that the methoxy group of **6** gave 50-fold and the methyl group threefold better binding. The electron-withdrawing carboxamide group of **13** had no effect on binding, but the chloro group of **3** gave a threefold increment in binding. Effects of substituents on the 3 position were smaller; note that the chloro group of **4**, the trifluoromethyl of **12**, the methoxy of **7**, and the amino group of **11** gave only about a 2–4-fold spread in binding.

If a benzene ring is complexed to an enzyme only by hydrocarbon interaction with an enzyme, then the phenyl binding should be influenced by the polarity of the substituents;[6] such a correlation has been previously noted with phenyl binding to dihydrofolic reductase where nonpolar substituents increased binding, but polar substituents decreased binding.[11-13]

A greater than 15-fold loss in the binding with the *p*-carboxyl group of **14**, which is fully ionized at the pH 7.4 of the assay, was noted; this result can be due to a repulsion of the hydrated carboxylate group either from a hydrophobic group or an anionic group on the enzyme.[12] If the latter anionic–anionic repulsion existed, then the *p*-amino group of **10** should have a strong affinity for this supposed enzymic anionic group. Since both the polar *p*-amino group of **10** and the polar carboxylate group of **14** are repulsed from the enzyme, this gives strong evidence that a hydrophobic region on

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

(2) For the previous paper on Irreversible Enzyme Inhibitors see B. R. Baker and M. A. Johnson, *J. Heterocyclic Chem.*, in press.

(3) For the previous paper on xanthine oxidase see B. R. Baker and J. Kozma, *J. Med. Chem.*, **10**, 682 (1967); paper XCV of this series.

(4) For the previous paper on guanine deaminase see 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) For a general discussion of modes of binding of inhibitors to enzymes see 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, Chapter II.

(7) See ref 6 (whole book).

(8) B. R. Baker, *J. Pharm. Sci.*, **53**, 347 (1964).

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

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

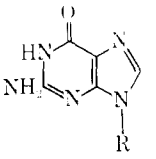
(11) See ref 6, Chapter X.

(12) B. R. Baker and B.-T. Ho, *J. Heterocyclic Chem.*, **2**, 335 (1965).

(13) E. Miller and C. Hansch, *J. Pharm. Sci.*, **56**, 92 (1967).

Table I

Inhibition[a] of Guanine Deaminase[b] and Xanthine Oxidase[c] by

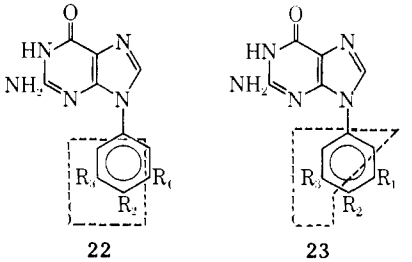


		Guanine deaminase		Xanthine oxidase	
Comod	R	μM concn for 50% inhib	$([I]/[S])_{0.5}$[d]	μM concn for 50% inhib	$([I]/[S])_{0.5}$
1	CH_3	275[e]	21	58[f]	7.1
2	C_6H_5	10[g]	0.75	0.41[f]	0.051
3	4-ClC_6H_4	3.8[e]	0.29	1.8[f]	0.22
4	3-ClC_6H_4	2.5	0.20	0.27	0.033
5	3,4-$Cl_2C_6H_3$	0.93	0.070	1.1	0.14
6	4-$CH_3OC_6H_4$	0.20	0.015	0.50	0.062
7	3-$CH_3OC_6H_4$	3.8	0.29	0.22	0.027
8	3,4-$(CH_3O)_2C_6H_3$	7.0	0.52	0.72	0.089
9	4-$(CH_3)_2NC_6H_4$	34	2.6	2.1	0.26
10[g]	4-$NH_2C_6H_4$	26	2.0	3.7	0.46
11[g]	3-$NH_2C_6H_4$	5.9	0.44	0.60	0.074
12	3-$CF_3C_6H_4$	4.5	0.34	0.15	0.018
13	4-$C_6H_4CONH_2$	13	1.0	0.42	0.052
14	4-$C_6H_4COO^-$	>200[h]	>15	0.12	0.015
15	4-$CH_3C_6H_4$	3.5	0.26	1.6	0.20
16	4-$C_2H_5C_6H_4$	1.3	0.10	0.68	0.084
17	3-$CH_3C_6H_4$	2.0	0.15	0.24	0.030
18	β-naphthyl	0.22	0.017	0.41	0.051
19	4-$C_2H_5OC_6H_4$	0.098	0.0074	0.11	0.014
20	$C_6H_5CH_2$	370[e]	28	23[f]	2.9
21	$C_6H_5(CH_2)_2$[i]	190	14	16	2.0

[a] The technical assistance of Pepper Caseria, Maureen Baker, and Barbara Baine 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 10% DMSO as previously described,[9] except that a double-beam Cary 11 or 15 spectrophotometer was used. [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.[10] [d] Ratio of concentrations of inhibitor to substrate giving 50% inhibition. [e] Data previously reported.[4] [f] Data previously reported.[5] [g] See ref 17 for synthesis. [h] No inhibition was observed at a concentration of 50 μM; since 20% inhibition is readily detected, the concentration for 50% inhibition is greater than four times the concentration measured. [i] Prepared according to general method in the Experimental Section; the physical properties agreed with those in ref 19, and analytical data were satisfactory.

the enzyme extends past the *para* position of the 9-phenyl group of 9-phenylguanine (**2**).

The proposition that the hydrophobic bonding region on guanine deaminase extends past the phenyl group of **2** when it is complexed to the enzyme then accounts for most of the observed binding of **3**–**19**. Diagramatically, this hydrophobic bonding region can be indicated by structure **22** in Figure 1. That the *p*-R_2 group is still in the hydrophobic region is strongly indicated by the relatively poor binding of polar substituents (**9**, **10**, **14**) in this area; conversely, this concept is supported by the threefold increment in binding by the *p*-methyl group of **15** and the *p*-chloro group of **3**, as well as an eightfold increment in binding by the *p*-ethyl group of **16**. That hydrophobic bonding can also occur at R_3 is indicated by the 2–4-fold increment in binding observed with the *m*-methyl group of **17**, the *m*-trifluoromethyl group of **12**, and the *m*-methoxy group of **7**. These effects at R_2 and R_3 can be combined in the β-naphthyl group of **18** which shows a 45-fold increment in binding over that of the phenyl group of **2**.



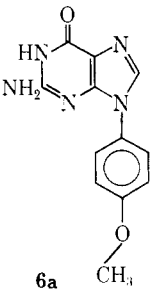
22 23

Figure 1.—Proposed hydrophoblic bonding region on guanine deaminase (**22**) and xanthine oxidase (**23**), as indicated by the dotted lines. The hydrophoblic region projected to the right, instead of to the left, of the benzene ring is equally possible at this time.

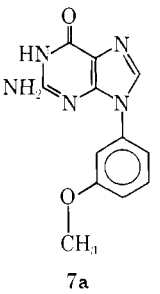
The R_1 group is thought to be outside of the hydrophobic binding area; whether R_1 is in a polar area of the enzyme or is simply not in contact with the enzyme cannot be stated at this time. This R_1 area is supported by the fact that the *m*-amino substituent of **11** causes little change in binding compared to **2**; however, 9-(*m*-aminophenyl)guanine (**11**) binds four times better than 9-(*p*-aminophenyl)guanine (**10**). Thus, a *meta*-substituted benzene could either complex to the enzyme with the substituent in the R_1 area if the substituent is polar (as in **11**) or at the R_3 area if the substituent is hydrophobic (as in **17** or **18**).

Of considerable interest is the 50-fold increment in binding observed with the *p*-methoxy substituent of **6** compared to the 2.6-fold increment with the *m*-methoxy substituent of **7** and practically no increment in binding with both substituents present, as in **8**. The binding of the *m*-methoxy substituent of **7** is not out of line with

the amount of hydrophobic bonding expected for a single methyl group; if the 3-methoxy group is in the plane of the benzene ring, as indicated in **7a**, then the contour is similar to that of the β-naphthyl of **18** where



6a



7a

strong hydrophobic bonding is seen. Similarly, it is logical to place the *p*-methoxy group in conformation **6a** when **6** is complexed to the enzyme. However, simple hydrophobic bonding by a single methyl can theoretically only give maximally a tenfold increment,[6,14] in contrast to the 50-fold increment observed with **6**; furthermore, there might be some repulsion of the ether oxygen from a hydrophobic region pictured at R_2 in **22**. If it is assumed that there is no repulsion of the ether oxygen, then the difference between the tenfold calculated increment with the *p*-methoxy substituent of **6** and the 50-fold increment observed has to be accounted for.

That the methyl group of **6** is most probably in a hydrophobic environment is indicated by the further increment in hydrophobic bonding by the *p*-ethoxy substituent of **19**. Two ways can be envisioned for the extra 5–10-fold increment observed with the *p*-methoxy substituent of **6**. (a) Although the ether oxygen is presumed to be in a hydrophobic region, some charge-transfer complex could exist between an ether oxygen and the phenyl of a phenylalanine group; the latter phenyl could also hydrophobically bond with the *p*-methyl substituent of **15**. (b) The *p*-methoxy substituent causes a conformational change in the enzyme that allows increased interaction of the phenyl group of **6** with the enzyme; such an explanation has been previously invoked to explain the increment in binding between *n*-propyl and *n*-butyl at position 1 of 4,6-diamino-1,2-dihydro-2,2-dimethyl-*s*-triazine when measured as inhibitors of dihydrofolic reductase.[14]

Further experiments are planned to differentiate between a and b; such experiments might even lead to a third explanation which would be more satisfactory than either a or b. Currently, explanation b is favored; if the ether oxygen of **6** were acting as an electron donor in a charge-transfer complex to the enzyme, then such a complex should also be possible with the *p*-ether group of 3,4-dimethoxyphenylguanine (**8**). Since **8** binds little differently from 9-phenylguanine (**2**), such an ether interaction is unlikely. That **8** loses the binding increments observed with the *m*-methoxy substituent of **7** and the *p*-methoxy substituent of **6** can be rationalized by consideration of **6a** and **7a** as the suggested conformations for optimum binding; the proton repulsion between the two methyl groups of **8** might not allow either methoxyl group to assume conformation **6a** or **7a**.

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

A chloro group at either the 3 (**4**) or 4 position (**3**) gave a 3–4-fold increment in binding; this increment is readily accounted for by a hydrophobic interaction of the chlorine atoms with the enzyme. Furthermore, the bonding by the two chlorine atoms is additive as seen by the binding of the 3,4-dichloro derivative (**5**); this additive binding by the chlorine atoms is in marked contrast to the loss in binding with the 3,4-dimethoxy derivative (**8**) which inhibits poorer than the monomethoxy derivatives (**6**, **7**).

Xanthine Oxidase.—No correlation between binding to xanthine oxidase and the Hammett σ value of substituents was observed (Table I), as explained with binding to guanine deaminase. The *p*-carboxylate group of **14** gave about a threefold increment in binding to the enzyme compared to **2**; since the *p*-amino group of **10** gave a ninefold loss in binding, it seems probable that the enzyme has an electron-poor hydrophilic group in this R_2 area as depicted in **23**. That this R_2 region (Figure 1) is hydrophilic is indicated by the 4–5-fold repulsion of the hydrophobic *p*-chloro group of **3** and the *p*-methyl group of **15**.

As indicated in Figure 1, the hydrophobic bonding region is proposed to include the R_3 area and part of the benzene ring. Note that hydrophobic substituents at the R_3 position such as 3-chloro (**4**), 3-methoxy (**7**), 3-trifluoromethyl (**12**), and 3-methyl (**17**) give 2–3-fold increments in binding. The polar 3-amino group of **11** shows a slight loss in binding and can be positioned in the R_1 area of **23**. Since the *p*-methyl group of **15** is repulsed from a polar region on the enzyme, the binding of the naphthyl group (**18**) should be compared with the case of **15**, where a fourfold difference occurs attributable to a hydrophobic interaction. Furthermore, the terminal methyl of the *p*-ethoxy group of **19** gives a fivefold increment in binding compared to **6**, indicating that the terminal methyl group is complexed to the enzyme in the dotted area of **23**.

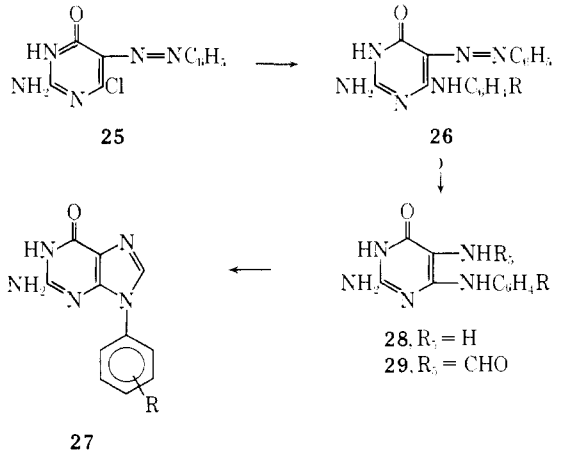
Comparison of Guanine Deaminase and Xanthine Oxidase.—The binding of the 9-phenyl group of 9-phenylguanine (**2**) to neither enzyme is influenced by electron-donating or electron-withdrawing groups. Binding to both enzymes is influenced by the relative hydrophobic character of substituents and the position of these substituents on the inhibitor. Particularly noteworthy is the loss in binding to guanine deaminase by the *p*-carboxyl group of **14**, but the gain in binding to xanthine oxidase with this substituent. Both enzymes show additional hydrophobic bonding at the *meta* position (see Figure 1) but also can tolerate polar *meta* substituents in a different conformation. Additional hydrophobic bonding to guanine deaminase can be obtained with *p*-methyl (**15**) and *p*-ethyl (**16**) substituents, but hydrophobic bonding to xanthine oxidase does not appear at the *para* position until the carbon is at least three atoms away from the benzene ring, as in **19**.

Further studies on the dimensions of the hydrophobic bonding regions of the two enzymes (see **22** and **23**, Figure 1) as well as conformational requirements for optimum binding (note **20** and **21** compared to **2**) are continuing; even more potent reversible inhibitors of guanine deaminase and xanthine oxidase should emerge than the compounds in Table I, some of which might be complexed 100 times better than the substrates. Another practical reason for knowing the dimensions of the

hydrophobic bonding region is to be able to position properly a leaving group on an inhibitor in order to convert it to an active-site-directed irreversible inhibitor;[6,8] if the leaving group is positioned in a hydrophobic bonding area, then irreversible inhibition is unlikely to occur due to lack of a nucleophilic group in a nonpolar area.[11,15,16] From Figure 1, logical positions for placement of a leaving group for irreversible inhibition of xanthine oxidase would be at R_1 or R_2 (**23**); for guanine deaminase, the only logical position yet available for placement of a leaving group would be the R_1 position of **22**. The results of such studies with the bromoacetamido group placed on the *meta* and *para* position of 9-phenylguanine (**2**) is the subject of paper CIII of this series.[17]

Chemistry.—The 9-arylguanines (**27**) of Table I were synthesized by modification of the general methods of Robins, *et al.*[18,19] The 5-phenylazo-6-chloropyrimidine (**25**)[20] was condensed with 1 equiv of the appropriate arylamine in refluxing ethanol to give **26**; this reaction was feasible even with weakly nucleophilic anilines substituted with electron-withdrawing groups. The fact that acid was generated in the reaction mixture suggests that it is the protonated species of the 6-chloropyrimidine (**25**) that reacted with the arylamine, since Maggioto and Phillips[21] have shown that nucleophilic displacement of the chloro group of another pyrimidine by arylamines is acid catalyzed. As Robins, *et al.*, observed,[18] the 6-arylamino-5-phenylazopyrimidines (**26**) appeared to decompose when attempts were made to purify them; however, after being thoroughly washed with alcohol the arylaminopyrimidines (**26**) moved as a single spot on tlc and had a typical visible spectral peak[18] near 425 mμ.

Reduction of the azo linkage of **26** to **28** with sodium hydrosulfite in alkaline solution[18] caused considerable difficulty. In some cases the compounds were insoluble in the alkaline solution and failed to reduce; when reduction was achieved, with or without an added solvent such as 2-methoxyethanol, the products were quite unstable to air, particularly when the benzene ring was substituted with an electron-donating group.



Since 5-formamidopyrimidines are considerably stabilized to air oxidation and since 5-formamidopyrimidines are readily prepared by reductive formylation of 5-nitrosopyrimidines with zinc in formic acid,[22] this method was investigated with the 5-phenylazo-6-arylaminopyrimidines (**26**). The reduction proceeded smoothly to give mainly **29**. In some cases tlc showed the presence of only one component (**29**), but in other cases, two other components were also present; one of these was a minor component of the desired purine (**27**) and the other was unformylated 5-aminopyrimidine (**28**). Since both **28** and **29** are converted to **27** on treatment with formamide containing some formic acid,[19] the intermediate crude **29** was not purified, but was converted directly to **27**. The ring closure of **29** to **27** was conveniently monitored by use of tlc or the uv spectral shift to lower wavelength[19] as the reactions proceeded.

This reaction sequence has so far been successful with all but two of the substituted 9-phenylguanines. The first case was **29** where R = *p*-formamido; the compound was too insoluble to react.[17] The second case was the *p*-cyano derivative of **29**. Cyclization of **29** (R = *p*-CN) in boiling formamide containing formic acid for the usual 5 hr gave a purine (**27**), but the cyano group had been converted to carboxamide; when the cyclization time was decreased to 3 hr, a mixture of the *p*-cyano and *p*-carboxamide derivatives of **27** were obtained. The *p*-carboxamide derivative of **27** was then prepared unequivocally from **25** by reaction with *p*-aminobenzamide.

Experimental Section

Melting points were determined in capillary tubes on a Mel-Temp block. Infrared spectra were determined in KBr pellet with a Perkin-Elmer 137B or 337 spectrophotometer. Ultraviolet spectra were determined in water with a Perkin-Elmer 202 spectrophotometer. Tlc was run on Brinkmann silica gel GF_{254}, unless otherwise indicated, and spots were detected by visual examination under ultraviolet light.

9-Arylguanines (27).—A mixture of 2.0 g (8 mmoles) of **25**, 8 mmoles of aromatic amine, and 25 ml of absolute ethanol was refluxed with stirring for 5 hr; during this period the product (**26**) gradually separated from solution. The cooled mixture was filtered and the product was washed with ethanol until the washings were light yellow. These intermediate 5-phenylazo-6-arylaminopyrimidines (**26**) showed maxima at pH 1 near 250 and 425 mμ with a ratio of about 1:1.3; these compounds (**26**) moved as a single spot on tlc in 25:1 $CHCl_3$–EtOH.

To a mixture of the crude **26** and 30 ml of boiling 97% formic acid was added zinc dust portionwise until the solution became colorless, then the mixture was gently boiled for an additional 25 min. The mixture was filtered and the precipitated zinc and zinc salts were washed with 90% formic acid. The combined filtrate and washings were spin-evaporated *in vacuo* to a thin syrup,[23] then the hot solution was diluted with H_2O (50 ml). The product was collected on a filter, then washed with H_2O. The wet filter cake of crude **29** was carried directly to the next step. These compounds were characterized by tlc in 3:5 EtOH–$CHCl_3$ and by their ultraviolet maxima near 287 mμ at both pH 1 and 13.

The crude 5-formamidopyrimidine (**29**) was suspended in 50 ml of formamide and 4 ml of 97% formic acid, then the mixture

(15) B. R. Baker and J. H. Jordaan, *J. Pharm. Sci.*, **55**, 1417 (1966); paper LXVII of this series.

(16) B. R. Baker and H. S. Shapiro, *ibid.*, **55**, 1422 (1966); paper LXVIII of this series.

(17) B. R. Baker and W. F. Wood, *J. Med. Chem.*, **10**, 1106 (1967).

(18) H. C. Koppel, D. E. O'Brien, and R. K. Robins, *J. Am. Chem. Soc.*, **81**, 3046 (1959).

(19) C. W. Noell and R. K. Robins, *J. Med. Pharm. Chem.*, **5**, 558 (1962).

(20) W. R. Boon and T. Leigh, *J. Chem. Soc.*, 1499 (1951).

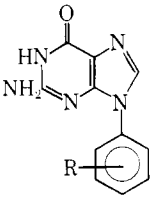
(21) A. Maggioto and A. P. Phillips, *J. Org. Chem.*, **16**, 376 (1951).

(22) B. R. Baker, J. P. Joseph, and R. E. Schaub, *ibid.*, **19**, 631 (1954).

(23) If the solution is evaporated to dryness, both the intermediate **29** and the final **27** contained zinc salts which were difficult to remove.

TABLE II

PHYSICAL CONSTANTS OF



Compd[a]	R	Yield,[b] %	Calcd, % C	Calcd, % H	Calcd, % N	Found, % C	Found, % H	Found, % N	λ_{max}, mμ pH 1	λ_{max}, mμ pH 13
4	3-Cl	32	50.5	3.08	26.8	50.6	2.87	27.0	272	269
5	3,4-Cl_2	22[c]	44.6	2.38	23.6	44.7	2.51	23.6	232, 272	245, 267[d]
6	4-CH_3O	19	56.0	4.31	27.2	56.0	4.32	27.1	231, 273[d]	230, 272
7	3-CH_3O	14	54.2[e]	4.54	26.3	54.4	4.38	26.2	275	273
8	3,4-$(CH_3O)_2$	18	54.4	4.56	24.4	54.2	4.75	24.4	250, 281	237, 273
9[f]	4-$(CH_3)_2N$	4	57.8	5.22	31.1	57.8	5.40	31.0	272	268
12	3-CF_3	15	48.8	2.73	23.7	48.8	2.91	23.5	268	269
13	4-$CONH_2$	15	52.8[g]	3.75	30.7	52.9	4.11	30.5	237, 270[d]	252[h]
14	4-COOH	35[i,j]	52.3[k]	3.47	25.4	52.0	3.79	25.2	252[h]	262, 278[d]
15	4-CH_3	16	59.7	4.60	29.0	59.5	4.81	28.8	262, 278[d]	269
16	4-C_2H_5	15	61.2	5.13	27.4	61.4	5.17	27.6	262[h]	270
17	3-CH_3	25	59.7	4.60	29.0	59.6	4.72	29.3	262[h]	268
18[l]	3,4-Benzo	23	65.0	4.00	25.3	64.8	3.96	25.5	278	270
19	4-C_2H_5O	12	57.6	4.83	25.8	57.7	4.97	26.0	231, 272[d]	266

[a] All compounds showed one spot on tlc in 3:5 EtOH–$CHCl_3$ unless otherwise indicated and had infrared spectra in agreement with their assigned structures; all of the compounds showed no mp below 300°. [b] Over-all yield from **25**. [c] The compound was purified by solution in 200 ml of hot 30% H_2SO_4, neutralization with NH_4OH, then filtration; the compound was then heated in 10% NaOH which gave an insoluble sodium salt that was collected, dissolved in hot 0.1 *N* NaOH, then precipitated by neutralization. [d] Inflection point. [e] Hemihydrate. [f] It was necessary to neutralize the dilute aqueous formic acid solution to precipitate the intermediate **29**. [g] Calculated with 1% H_2O. [h] The strong extinction of the substituted benzene peak masked the longer wavelength peak, but the presence of the latter was notable as a bulge on the long-wavelength side of the major peak. [i] Yield based on **29**; over-all yield from **25** was 15%. [j] No suitable solvent for tlc or paper chromatography could be found; the compound was purified by solution in hot 0.1 *N* NaOH, then acidification to near pH 3 with HCl. [k] Calculated with $0.25H_2O$. [l] A 9-naphthylguanine has been described in the literature,[18] but it was not specified whether it was α or β.

was refluxed 4–5 hr. The solution was poured into 100 ml of ice H_2O and filtered. The crude purine (**27**) was washed with water. The moist cake was dissolved in 50–100 ml of 6 *N* aqueous HCl, the solution was clarified with decolorizing carbon, then neutralized to about pH 7 with concentrated NH_4OH. The collected purine was washed with water, then the moist cake was dissolved in boiling 15% NH_4OH. The solution was clarified with decolorizing carbon, then neutralized with 90% formic acid. The purine (**27**) was collected, washed with water, then dried in high vacuum at 100° over P_2O_5. If HCl was used for neutralization, it was difficult to remove occluded NH_4Cl; in contrast, occluded ammonium formate is volatile under the 100° high-vacuum drying conditions. See Table II for compounds made in this manner. An exception is **29** where R = *p*-COOH, which is described below.

The cyclization reaction could be followed by the uv shift to about 20-mμ shorter wavelength. The reaction could also be monitored by tlc in 3:5 EtOH–$CHCl_3$.

2-Amino-6-(*p*-carboxyanilino)-5-formamido-4-pyrimidinol (29) (R = *p*-COOH).—Crude **26** (R = *p*-COOH) was obtained in 71% yield from **25** by the general method above; a mixture of 1.7 g of this crude **26** with 10 ml of 90% formic acid was heated on a steam bath. Zinc dust was added portionwise until the red color had bleached to white. After being heated on the steam bath for another 30 min, the mixture was filtered. The precipitate contained most of the product; the filtrate was processed by the general method above. The zinc precipitate was extracted with concentrated NH_4OH. The filtered solution was acidified to about pH 3 and additional product was collected. The two fractions were combined since they had the identical mobility on tlc on cellulose powder with 40:30:15:15 *t*-butyl alcohol–butanone–concentrated NH_4OH–H_2O; yield 1.1 g (71%). For analysis the compound was dissolved in 0.1 *N* aqueous NaOH, then the solution was clarified with decolorizing carbon. Acidification with HCl gave a white solid that was collected and thoroughly washed with water; λ_{max} (pH 1), 308; (pH 13), 267, 320 mμ.

Anal. Calcd for $C_{12}H_9N_5O_3 \cdot H_2O$: C, 46.9; H, 4.26; N, 22.8. Found: C, 46.5; H, 4.27; N, 22.7.