130326-23-7; **13**, 130350-27-5; **14**, 127837-47-2; **16**, 122539-76-8; **19**, 130326-24-8; **20** (M = Si, $n$ = 6, $R_3$ = $PhMe_2$, X = H), 116488-00-7; **20** (M = Sn, $n$ = 2, R = Bu, X = OH), 107399-01-9; **20** (M = Sn, $n$ = 2, R = Me, X = OH), 76077-09-3; **21** (M = Si, $n$ = 6, $R_3$ = $PhMe_2$, X = H), 87437-03-4; **21** (M = Sn, $n$ = 2, R = Bu, X = OH), 122229-78-1; **21** (M = Sn, $n$ = 2, R = Me, X = OH), 76077-30-0; $(PhMe_2Si)_3CuLi_2$, 122343-28-6; $Me_4Sn$, 594-27-4; $H(CH_2)_6C{\equiv}CH$, 629-05-0; $HO(CH_2)_2C{\equiv}CH$, 927-74-2; $^{119}Sn$, 14314-35-3; $^{29}Si$, 14304-87-1; MeCu(CN)Li, 41753-78-0; CuCN, 544-92-3; 2-cyclohexenone, 930-68-7; 3-(dimethylphenylsilyl)-cyclohexanone, 67262-98-0; 3-(tributylstannyl)cyclohexanone, 63831-51-6; 3-(trimethylstannyl)cyclohexanone, 63831-50-5.

# Regioselective Synthesis of Imidazo[4,5-*g*]quinazoline Quinone Nucleosides and Quinazoline Amino Nucleosides. Studies of Their Xanthine Oxidase and Purine Nucleoside Phosphorylase Substrate Activity

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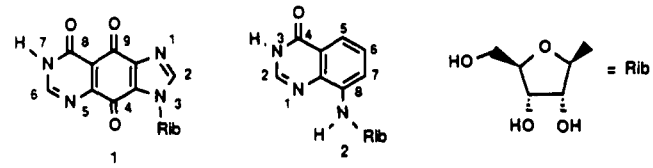
The regioselective synthesis of 3-ribofuranosylimidazo[4,5-*g*]quinazoline-4,8,9(3*H*,7*H*)-trione (**1**) (benzoquinone-stretched-out inosine) and 8-(ribofuranosylamino)quinaozlin-4(3*H*)-one (**2**) was carried out in conjunction with the design of reductive alkylating nucleosides and new purine nucleoside mimics, respectively. The preparation of **1** was carried out by regioselective ribosylation of 4-nitroimidazo[4,5-*g*]quinazolin-8(3*H*,7*H*)-one (**3**) followed by nitro group reduction, Fremy oxidation, and deacetylation. Regiocontrol of ribosylation has steric origions: the 4-nitro group of **3** directs silylation to the N(1) position, which results in ribosylation exclusively at the N(3) position under Vorbrüggen reaction conditions. Regiocontrol during the preparation of **2** was possible by generating a stabilized ribofuranosyl carbocation, which selectively reacts with the amine group of the base. Nucleoside **1** is a purine-like quinone by virtue of its oxidation by xanthine oxidase. The potential inosine mimic **2** does not undergo phosphorolysis by purine nucleoside phosphorylase (PNPase), but the base form (8-aminoquinazolin-4(3*H*)-one) does bind to the PNPase active site as tightly as hypoxanthine. Factors which contribute to this binding behavior are discussed.

Imidazo[4,5-*g*]quinazolines and quinazolines can mimic purines in a number of enzymatic systems. Leonard and co-workers[2] found that imidazo[4,5-*g*]quinazolines, and their nucleoside and nucleotide derivatives, act as substrates and cofactors for certain purine-utilizing enzymes. Work in this laboratory showed that quinone analogues of imidazo[4,5-*g*]quinazolines can be functionalized as enzyme-directed reductive alkylating agents.[3-5] Like many naturally occurring reductive alkylating agents,[6] quinone reduction is followed by leaving group elimination to afford an alkylating quinone methide species. Finally, quinazolines are substrates for the purine-utilizing enzyme xanthine oxidase,[7] and it was possible to design quinazoline-based reductive alkylating agents of this enzyme.[8]

The findings cited above have prompted investigations of nucleoside reductive alkylating agents based on imidazo[4,5-*g*]quinazolines and of purine nucleoside mimicks based on aminoquinazolines. These investigations required the efficient regioselective ribosylation of these ring systems. Described herein are the regioselective ribosylation studies which led to the synthesis of the nucleosides in Chart I and the results of enzyme binding studies with xanthine oxidase and purine nucleoside phosphorylase.

**Chart I**




1 2

The synthetic methodologies employed to prepare nucleoside **1** could be applied to the preparation of analogues bearing a leaving group (i.e., reductive alkylating agents). Enzymatic studies with **1** indicate it is oxidized by xanthine oxidase. Amino nucleoside **2** was designed as an inosine mimic, wherein the ribofuranosyl and fused pyrimidone groups are in nearly the same relative positions as found in the inosine. Nucleoside **2** weakly binds to the active site of purine nucleoside phosphorylase (PNPase), but it is not a substrate. The free base of **2**, 8-aminoquinazolin-4-(3*H*)-one, is a good inhibitor of PNPase, however. A mechanism is presented for PNPase binding by the free base.

## Results and Discussion

**Nucleoside Reductive Alkylating Agents.** The strategy for preparing imidazo[4,5-*g*]quinazoline quinone nucleosides was to carry out regioselective ribosylation of **3** in Scheme I and then elaborate the quinone moiety by reduction of the nitro group and Fremy oxidation of the resulting amine. Leonard and co-workers have prepared structurally related nucleosides by mercury-catalyzed ribosylation of the imidazo[4,5-*g*]quinazoline ring[9] as well

(1) National Institutes of Health research career development award recipient 1988-1993 (CA 01349).
(2) Leonard, N. J. *Acc. Chem. Res.* **1982**, *15*, 128.
(3) Lee, C.-H.; Gilchrist, J. H.; Skibo, E. B. *J. Org. Chem.* **1986**, *51*, 4787.
(4) Lee, C.-H.; Skibo, E. B. *Biochemistry* **1987**, *26*, 7355.
(5) Lemus, R. H.; Lee, C.-H.; Skibo, E. B. *J. Org. Chem.* **1989**, *54*, 3611.
(6) Moore, H. W.; Czerniak, R. *Med. Res. Rev.* **1981**, *1*, 249.
(7) Skibo, E. B.; Gilchrist, J. H.; Lee, C.-H. *Biochemistry* **1987**, *26*, 3032.
(8) Lemus, R. H.; Skibo, E. B. *J. Org. Chem.* **1988**, *53*, 6099.

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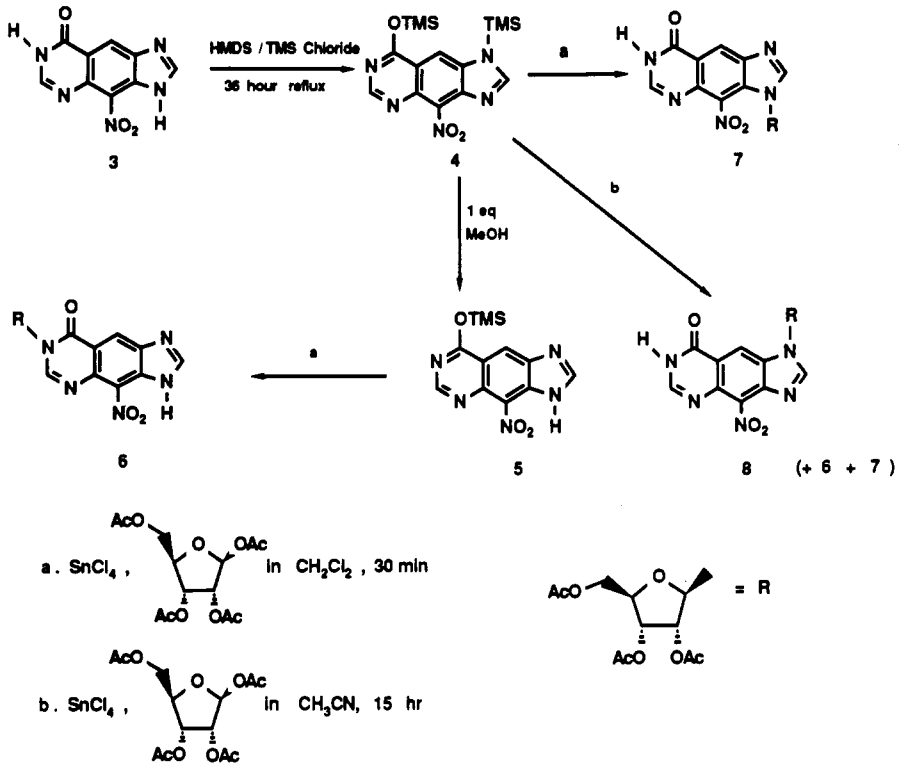
**Scheme I**




as by ribosylation of a benzimidazole derivative followed by pyrimidone ring annelation.[10] In contrast to these previous efforts, the synthetic methods described below permitted ribosylation of the 4-nitroimidazo[4,5-g]-quinazoline derivative **3** to afford single products.

The unequivocal identification of ribosylation products was possible from NOE difference measurements, $pK_a$ measurements, and UV–visible spectral studies. Discussed first are the synthetic studies followed by discussions of the identification studies and the regioselectivity of the reactions.

**Imidazoquinazoline Quinone Synthesis.** Persilylation of **3** by treatment with refluxing 1,1,1,3,3,3-hexamethyldisilazane (HMDS) and trimethylsilyl chloride for a 36-h period afforded the 8-*O*-silyl, *N*(1)-silyl derivative **4**. The silylated fused imidazole ring is more reactive toward ribosylation than the silylated fused pyrimidine ring. Thus, the ribosylation of **4** employing the reaction conditions a in Scheme I afforded the N(3) nucleoside **7**. The N(1) and N(7) isomers, **8** and **6**, respectively, are formed in only trace amounts. Treatment of **4** with 1 equiv of methanol removed the more reactive silyl group to afford **5**. Ribosylation of **5** employing the reaction conditions a in Scheme I afforded the N(7) nucleoside **6** as the major product. The N(1)-nucleoside **8** was required for comparative spectral and enzymatic studies. This isomer could be prepared only as a mixture by ribosylation in acetonitrile, conditions b in Scheme I.

Shown in Scheme II are the steps which led to the quinone nucleosides **1**, **15**, and **16**. Dithionite reduction of the nitronucleosides in Scheme I afforded the corresponding amine derivatives, which were oxidized to the acetylated quinone nucleosides employing Fremy's salt.[11]

**Table I. NOE Difference Results for the Nitronucleosides**

| nucleoside | irradiated proton (δ) | enhanced proton (δ) | % NOE |
|---|---|---|---|
| 6 | aromatic (8.74) | –[a] | |
| | aromatic (8.58) | – | |
| | aromatic (8.49) | H(1′) | 3.7 |
| | H(1′) | aromatic (8.49) | 3.2 |
| 7 | aromatic (8.91) | H(1′) | 1.5 |
| | aromatic (8.58) | – | |
| | aromatic (8.17) | – | |
| | H(1′) | aromatic (8.91) | 2.7 |
| 8 | aromatic (8.99) | H(1′) | 4.3 |
| | aromatic (8.75) | H(1′) | 4.3 |
| | | H(2′) | 3.3 |
| | aromatic (8.19) | – | |
| | H(1′) | aromatic (8.99) | 3.7 |
| | | aromatic (8.75) | 2.6 |

[a] No NOE difference observed.

Oxidation of the N(7)-ribosylated amine derivative **9** to **12** proceeded with facility. In contrast, the N(1) and N(3) isomers (**11** and **10**, respectively) were difficult to oxidize perhaps due to steric interactions between the ribofuranosyl group and the oxidizing agent. Finally, acetate removal in NaOH/methanol afforded the deacetylated nucleosides.

**Identification Studies.** Consideration of NOE difference results, $pK_a$ measurements, and UV–visible spectral studies together provide unequivocal structural assignments of the nucleosides in Schemes I and II.

The NOE differences for the nitronucleosides in Table I permit assignment of **8** (and hence **11**, **14**, and **16** in Scheme II) as the N(1)-nucleoside. Of the three nucleoside isomers, only the N(1)-nucleoside could show NOEs between ribofuranosyl protons and two aromatic protons. Nucleosides showing NOEs with one aromatic proton (**6** and **7**) can either be assigned as the N(3)- or N(7)-ribosylated derivatives.

The N(3)- and N(7)-ribosylated derivatives were distinguished based on spectrophotometric $pK_a$ values of the

(9) Leonard, N. J.; Sprecker, M. A.; Morrice, A. G. *J. Am. Chem. Soc.* **1976**, *98*, 3987.
(10) Keyser, G. E.; Leonard, N. J. *J. Org. Chem.* **1979**, *44*, 2989.
(11) Zimmer, H.; Lankin, D. C.; Horgan, S. W. *Chem. Rev.* **1971**, *71*, 229.

**Scheme II**

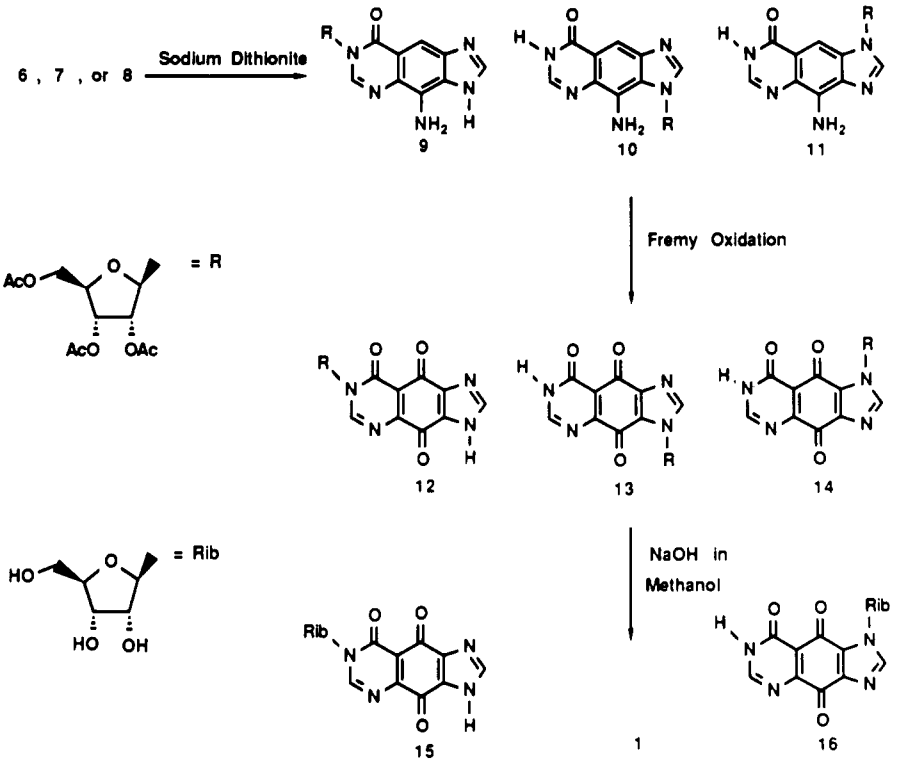


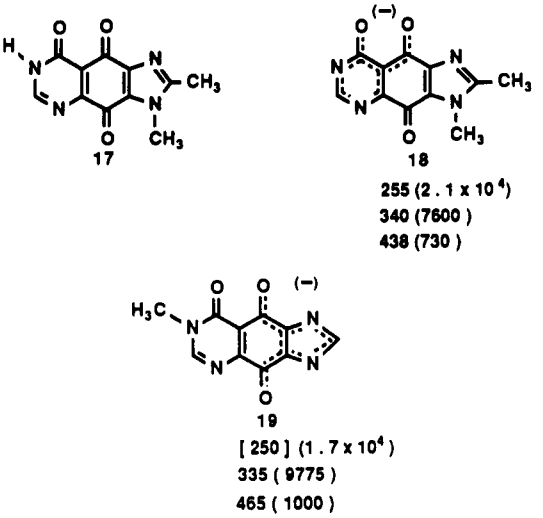

**Table II. Quinone Nucleoside Acid Dissociations**

| nucleoside | dissociated proton | $pK_a$ | UV–vis, nm (ε) |
|---|---|---|---|
| 15 | N(3) or N(1) | 7.52 ● 0.04 | 15: 325 (8300)<br>400 (1200)<br>15⁻: 255 (2 × 10⁴)<br>330 (1.2 × 10⁴)<br>470 (830) |
| 1 | N(7) | 5.83 ± 0.04 | 1: 320 (9200)<br>400 (840)<br>1⁻: 255 (2.0 × 10⁴)<br>327 (6300)<br>430 (1200) |
| 16 | N(7) | 5.74 ± 0.08 | 16: 320 (8200)<br>400 (850)<br>16⁻: 250 (2 × 10⁴)<br>320 (5000)<br>425 (1600) |

ribosylated quinone derivatives (Table II). Previous studies showed that the N(3)-substituted imidazo[4,5-*g*]-quinazoline-4,9-dione **17** possesses a low $pK_a$ value (6.15)[3] for N(7)-acid dissociation due to formation of a delocalized anion in the electron-deficient pyrimidine and benzoquinone rings (**18** in Chart II). In contrast, acid dissociation of the N(1) or N(3) proton from the N(7)-methyl imidazo[4,5-*g*]quinazoline derivative (**19** in Chart II) affords an anion destabilized by the electron-rich fused imidazole ring, $pK_a$ for **19H** ⇄ **19** + $H^+$ is 7.49 ± 0.05. Thus, the N(1)- and N(3)-ribosylated quinones should possess $pK_a$ value of ~6, while the N(7)-ribosylated derivative should possess a $pK_a$ value of ~7.5. Table II shows that the N(1)- and N(3)-ribosylated quinone derivatives (**16** and **1**) possess $pK_a$ values of 5.74 and 5.83, respectively. The N(7)-ribosylated derivative **15**, on the other hand, possesses a $pK_a$ value of 7.52.

The quinone nucleoside structural assignments were further confirmed by UV–visible comparisons. The UV–visible spectrum of **18**[3] in Chart II is similar to those of the structurally analogous nucleoside anions **1**⁻ and **16**⁻ in Table II. Likewise, the UV–visible spectrum of **19** in Chart II is similar to that of the nucleoside anion **15**⁻ in Table II. These data indicate that the change from an anion delocalized in the fused pyrimidine ring (**18**, **1**⁻, and **16**⁻) to one delocalized in the fused imidazole ring (**19** and **15**⁻) is associated with a red shift of only the highest $\lambda_{max}$ value (430–470 nm).

**Chart II**



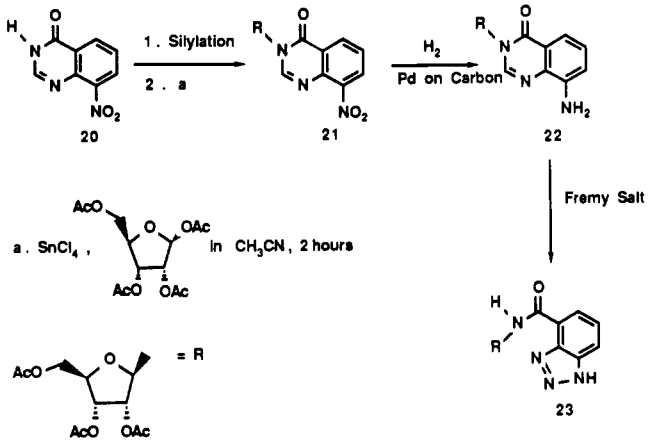

The reaction conditions shown in Scheme I generally result in formation of the β-nucleoside.[12] Confirmation of the β-configuration of **1** was obtained from an NOE difference study (Table III). NOE interactions between H(1′) and H(4′), as well as the absence of interactions between H(1′) and H(5′), are consistent with a β-nucleoside. Also consistent with a β-nucleoside are the NOE interactions between the H(2) and both H(2′) and H(3′).

(12) Vorbrüggen, H.; Nieballa, V.; Krolikiewicz, K.; Bennua, B.; Höfle, G. In *Chemistry and Biology of Nucleosides and Nucleotides*; Harmon, R. E., Robins, R. K., Townsend, L. B., Eds.; Academic Press: New York, 1978; p 251. (b) Vorbrüggen, H.; Höfle, G. *Chem. Ber.* **1981**, *114*, 1256.

**Table III. NOE Differences in the Ribofuranosyl Group of 1**

| irradiated proton | enhanced proton | % NOE |
|---|---|---|
| H(2) | H(1′) | 0.9 |
| | H(2′) | 1.5 |
| | H(3′) | 0.7 |
| | H(4′) | <0.1 |
| H(1′) | H(2) | 0.8 |
| | H(2′) | 1.3 |
| | H(4′) | 0.8 |
| | H(5′)a | 0.0 |
| | H(5′)b | 0.0 |
| H(4′) | H(1′) | 1.0 |
| | H(5′)a | 2.0 |
| | H(5′)b | 2.0 |
| H(5′)a | H(4′) | 1.7 |
| | H(5′)b | 6.3 |
| | H(2) | 0.4 |
| | H(1′) | 0.0 |
| H(5′)b | H(4′) | 1.1 |
| | H(5′)a | 5.2 |
| | H(2) | 0.3 |
| | H(1′) | 0.0 |

**Scheme III**



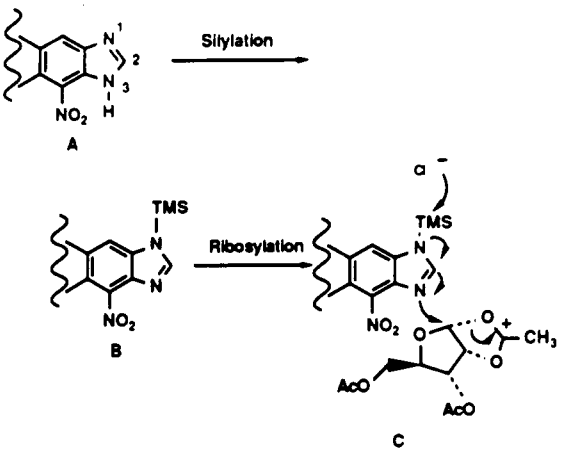

The NOE interactions noted above have also been used by Garner and Ramakanth[13] to assign the β-configuration to a guanine nucleoside.

**Regioselectivity.** The formation of the N(7)-nucleoside **6** from **5** is consistent with other quinazoline ribosylation studies. Stout and Robins[14] observed that ribosylation of quinazolin-4(3*H*)-one occurs only at the N(3)-position (analogous to the N(7)-position of the imidazo[4,5-*g*]-quinazoline system). Likewise, our ribosylation studies with the nitroquinazoline **20** affords only the N(3) isomer **21**. The identification of this isomer was possible through the synthetic sequence outlined in Scheme III. Reduction of **21** to **22** with palladium on charcoal/$H_2$ and then diazotization with Fremy's salt[15] affords the 1,2,3-benzotriazole nucleoside **23**.[16] $^1H$ NMR studies of **23** indicate the C(1′) proton is coupled to the amide proton. Quantitative $^{13}C$ NMR studies confirmed the carbon content of deacetylated **23** (see the Experimental Section).

The absence of N(1)-nucleoside formation in the reaction **4** → **7** is explained in conjunction with eqs 1 and 2. Fused imidazole ring silylation probably occurs at N(1) so as to avoid unfavorable steric interactions with the 4-nitro group.

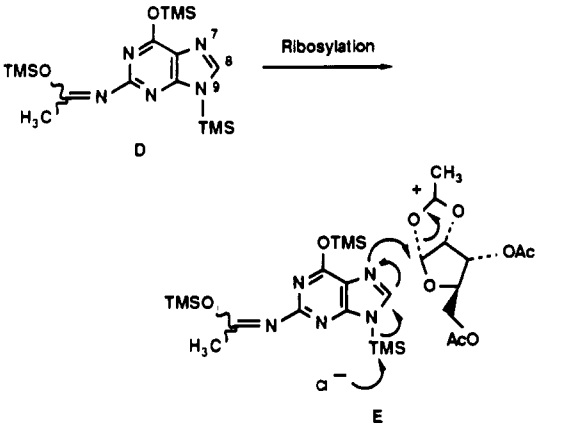
(13) Garner, P.; Ramakanth, S. *J. Org. Chem.* **1988**, *53*, 1294.
(14) Stout, M. G.; Robins, R. K. *J. Heterocycl. Chem.* **1969**, *6*, 89.
(15) Fremy's salt oxidizes electron-rich aromatic amines to quinones, but converts electron-deficient amines (i.e., aminoquinazolines) to diazonium salts.
(16) 1,2,3-Benzotriazole formation from diazotized **22** involves trapping of the diazonium salt by the N(1)-position followed by hydrolysis of the fused pyrimidone ring. For another example see ref 3.

Ribosylation by the mechanism proposed by Verbrüggen, as shown in structure C of eq 1, would then afford the




N(3)-ribosylated derivative as the kinetic product. However, thermodynamic reaction conditions should favor the less sterically congested N(1)-ribosylated product. Indeed, the ribosylation of **4** under thermodynamic reaction conditions (long reaction time in a polar solvent) afforded the N(1)-ribosylated product **8**.

Results of previous regioselective ribosylation studies of guanine[13] could also be interpreted in terms of steric control. Persilylation of N-acetylated guanine very likely affords D in eq 2. Silylation would occur at the N(9)-




position rather than at the N(7)-position so as to avoid steric interactions between the silyl groups. Ribosylation under kinetic conditions affords the N(7)-ribosylated derivative as the major product, presumably by the process shown in structure E. However, under thermodynamic conditions the more stable N(9)-ribosylated derivative is favored.

**Amino Nucleoside Synthesis.** Synthesis of the quinazoline and imidazo[4,5-*g*]quinazoline amino nucleosides (**2** and **26**, respectively) are discussed in conjunction with Scheme IV. Regiocontrol was achieved by employing 1-acetyl-2,3,5-tri-*O*-benzoylribofuranose as the ribosylating agent. The reversible nature of these reactions always resulted in a mixture of product and starting materials, however. The stabilized carbocation intermediate selectivity reacts with the amino group. On the other hand, the less stable carbocation arising from tetra-*O*-acetylated ribofuranose reacts with ring nitrogens as well as the amino group. Unambigous identification of the ribosylated product as an amino nucleoside was possible by $^1H$ NMR: the anomeric proton presents as a doublet of doublets which collapses to a doublet upon addition of $D_2O$.

The hydrolytic stability of the amino nucleoside appears to decrease as the basicity of the ribosylated nitrogen in-

Scheme IV

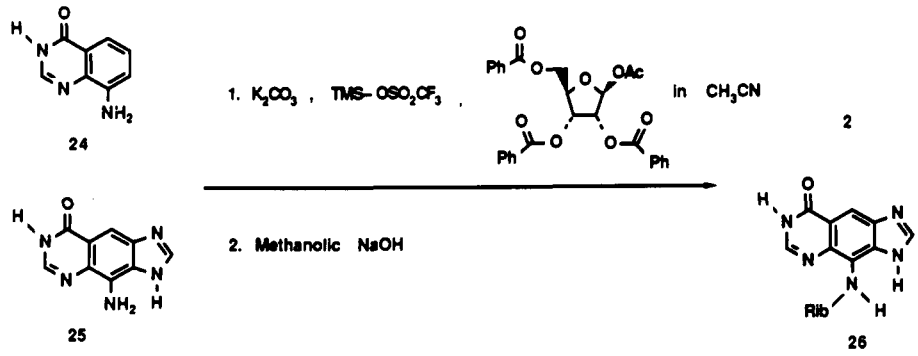
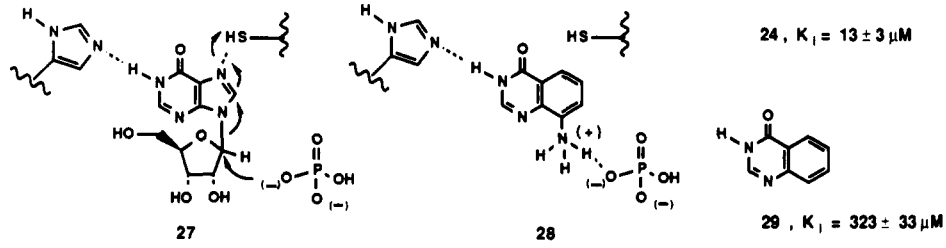



Chart III




creases. The electron-deficient quinazoline amino nucleoside **2** thus hydrolyses to the base slowly in pH 7.0 phosphate buffer, $t_{1/2}$ = 1.6 days. The amide nucleoside **23** is even stable in strong acid. An example of a stable naturally occurring amino nucleoside is clitosine,[17] which is an N-ribosylated diamino nitropyrimidine. In contrast to these examples, the electron-rich amino nucleoside **26** deribosylates rapidly in water and cannot be isolated.

**Enzyme Substrate Studies.** The substrate activity of the nucleosides prepared herein was assessed in two enzyme systems, xanthine oxidase and purine nucleoside phosphorylase.

Leonard and Keyser found that imidazo[4,5-*g*]-quinazoline-8(3*H*,7*H*)-one and the N(3)-ribosylated derivative were both oxidized by xanthine oxidase.[10] We have found that the quinone derivative of the first compound mentioned above (**17**) is also a substrate for xanthine oxidase.[3] As expected, the quinone analogue (**1**) of Leonard's N(3)-ribosylated derivative is rapidly oxidized by this enzyme.[18] Neither the N(7)- nor N(1)-ribosylated quinones (**15** and **16**, respectively) are substrates for the enzyme, however.

The aminoquinazoline derivative **24** is a weak substrate for xanthine oxidase: $k_{cat}$ = 4.9 min$^{-1}$ and $K_m$ = 7.98 × 10$^{-5}$ M. The aminoimidazo[4,5-*g*]quinazoline derivative **25** is likewise a weak substrate for the enzyme: $k_{cat}$ = 12.7 min$^{-1}$ and $K_m$ = 6.6 × 10$^{-5}$ M. These findings are consistent with our previous studies of substitutent effects on xanthine oxidase mediated oxidation of quinazolines.[7] Electron-rich substrates such as **24** and **25** are oxidized slowly due to slow nucleophile transfer to the substrate in the active site. In contrast, an electron-deficient derivative such as 6-chloroquinazolin-4(3*H*)-one is oxidized rapidly: $k_{cat}$ 1663 min$^{-1}$ and $K_m$ = 2.27 × 10$^{-5}$ M.[7] Ribosylated **24** (**2**) is not a substrate for xanthine oxidase at all, perhaps due to the unfavorable steric influence of the ribofuranosyl group on active site binding.

Purine nucleoside phosphorylase (PNPase) catalyzes the reversible $S_N2$ phosphate displacement of a purine base from the corresponding nucleoside. A postulated model of the PNPase active site is shown in structure **27** of Chart III.[19] Phosphate is bound to the active site, presumably by salt interactions with a lysine or arginine residue. The imidazole ring of a histidine residue hydrogen bonds with the fused pyrimidone ring and a sulfhydryl residue protonates the N(7)-position of the purine base.

PNPase substrate studies with **24** in the presence of ribose-1-phosphate revealed the absence of nucleoside **2** formation. Likewise, PNPase did not cleave **2** to **24** and ribose-1-phosphate. Molecular modeling both of the natural substrate (inosine) and **2** was carried out, and the pyrimidone rings of the energy-minimized structures were superimposed. The geometry of the amino group of **2** resulted in nonsuperimposable ribofuranosyl groups in these structures. It was noted that the amino group of **2** is placed at the C(1′) position of inosine when the pyrimidone rings are superimposed. The first observation may explain the lack of PNPase substrate activity by **2**. The second observation suggested that the amino group of **2** could interact with enzyme-bound phosphate as shown in structure **28** (Chart III).

PNPase inhibition studies of **24** and **29** were carried out by employing the coupled xanthine oxidase method.[20] The enzymatic phosphorolysis of inosine to hypoxanthine was followed by measuring the increase in absorbance at 292 nm accompanying the xanthine oxidase mediated oxidation of hypoxanthine to uric acid. As shown in Chart III, the 8-aminoquinazolinone **24** is a much better competitive inhibitor of PNPase than the unsubstituted analogue **29**. The lower $K_i$ of **24** is probably due to interactions of the amine with enzyme-bound phosphate. In fact, our pre-

(17) Moss, R. J.; Petrie, C. R.; Meyer, R. B.; Nord, L. D.; Willis, R. C.; Smith, R. A.; Larson, S. B.; Kini, G. D.; Robins, R. K. *J. Med. Chem.* **1988**, *31*, 786.

(18) Combination of **1** with xanthine oxidase in 0.05 M pH 7.4 phosphate buffer results in a new UV–visible spectrum, $\lambda_{max}$, nm ($\epsilon$): 450 (2000), 316 (1.2 × 10$^4$), 260 (1.5 × 10$^4$). The expected product, 3-ribofuranosylimidazo[4,5-*g*]quinazoline-2,4,6,8,9(1*H*,3*H*,5*H*,7*H*)-pentone, was observed by TLC but not isolated.

(19) Carlson, J. D.; Fischer, A. G. *Biochim. Biophys. Acta* **1979**, *571*, 21.

(20) Kalckar, H. M. *J. Biol. Chem.* **1947**, *167*, 429.

(21) Parks, R. E., Jr.; Stoeckler, J. D.; Cambor, C.; Savarese, T. M.; Crabtree, G. W.; Chu, S.-H. In *Molecular Actions and Targets for Cancer Chemotherapeutic Agents*; Sartorelli, A., Lazo, J. S., Bertino, J. R., Eds.; Academic Press: New York, 1981; p 229 (see Table II).

liminary results indicate the $K_i$ of **24** is dependent on phosphate concentration while the $K_i$ of **29** is not.

The $K_i$ of **24** is nearly the same as the $K_i$ for hypoxanthine (17 $\mu$M) for guanosine phosphorylysis.[22] This observation indicates that active-site interactions of the fused pyrimidone ring of **24**, together with interactions of the 8-amino group with phosphate, duplicate the strength of hypoxanthine binding to the PNPase active site. To assess the nature of the active-site interactions of the fused pyrimidone ring of **24**, comparisons were made between 2-substituted analogues of both **24** and hypoxanthine. Substitution of a 2-amino group on hypoxanthine (guanine) decreases the $K_i$ to 5 $\mu$M.[22] The 2-amino analogue of **24** (2,8-diaminoquinaozline-4(3*H*)-one) has a $K_i$ slightly higher than **24** (33 $\mu$M). The 2-hydroxy analogue of hypoxanthine (xanthine) also does not bind appreciably to the PNPase active site,[22] and the same analogue of **24** (8-aminoquinazoline-2,4(1*H*,3*H*)-dione) does not bind to the active site. This parallel binding behavior suggests that the quinazolinone ring and the natural purine substrates bind to the PNPase active site in the same fashion.

## Conclusions

Preparation of the desired 3-ribosylated imidazo[4,5-g]quinazoline-4,8,9(3*H*,7*H*)-trione **1** was possible by regioselective ribosylation of the 4-nitroimidazo[4,5-g]-quinazoline derivative **3** followed by nitro group reduction and Fremy oxidation to the quinone (Schemes I and II). Regioselectivity is thought to result from steric interactions. Silylation occurs at the N(1)-position so as to avoid steric interactions with the nitro group. The N(1)-silyl derivative then affords the N(3)-ribofuranosyl derivative under Verbrüggen reaction conditions. Steric interactions may likewise be responsible for the regioselectivity observed in the ribosylation of *N*-acetylguanine.

Quinone **1** is purine-like judging from its substrate activity with xanthine oxidase. Currently, nucleoside reductive alkylating agents (**1** functionalized with a leaving group) are being prepared for evaluation in enzymatic systems.

Regioselective synthesis of the quinazoline amino nucleoside **2** was possible employing 1-acetyl-2,3,5-tri-*O*-benzoylribofuranose as the ribosylating agent. The stablized ribofuranosyl carbocation is selectively trapped by the amino group. The quinazoline amino nucleoside (**2**) is not an inosine mimic, and PNPase phosphorolysis was not observed. The free base of **2** (**24**) binds to the PNPase active site as tightly as hypoxanthine, however. The fused pyrimidone ring of **24** binds to the active site in nearly the same way as natural purine substrates while the amino group of **24** interacts with enzyme-bound phosphate.

It has been assumed that only derivatives of the purine ring could be PNPase inhibitors.[22] Indeed known inhibitors of the enzyme are either purines or purine-like derivatives (e.g. triazoles).[23] This study shows that quinazoline derivatives could also bind to the active site well. Currently, we are developing quinazoline-based PNPase inhibitors.

## Experimental Section

All analytically pure compounds were dried under high vacuum in a drying pistol heated with refluxing methanol. Some of the compounds still contained water of crystallization that was determined from the elemental analysis found. Experimental nitrogen percentages for **6**, **7**, and **8** deviated from theoretical percentage by >0.5%. Repeat nitrogen analyses often showed a wide variation in percentage values; we believe this is due to incomplete combustion. $^1$H NMR and mass spectra support the assigned structures, however.

Uncorrected melting and decomposition points were determined with a Mel-Temp apparatus. Typically, the decomposition point of nucleoside derivatives was characterized by color darkening without complete melting. All TLC was run with Merck silica gel 60 ($F_{254}$) plates, employing a variety of solvents. IR spectra were taken as KBr pellets or thin films on KBr; the strongest IR absorbances are reported. Routine $^1$H NMR spectra were obtained on a 300-MHz instrument, and chemical shifts are reported relative to TMS. UV-visible spectral studies were carried out on a Perkin-Elmer 559 spectrophotometer.

NOEs were determined in dimethyl-$d_6$ sulfoxide on a 400-MHz instrument. The alternating on and off resonance irradiation times varied from 0.3 to 0.9 s. The percent NOEs were calculated as follows: area of the difference signal × 100/area of the off-resonance signal.

Molecular modeling was carried out with the Polygen Charm & Quanta programs on an IRIS 4D80.

p$K_a$ constants were determined by spectrophotometric titration in $\mu$ = 1.0 (KCl) aerobic aqueous solvent with a Perkin-Elmer 559 spectrophotometer. Absorbance vs pH data were computer fit to abs = $(A_T a_H \epsilon_{HA} + A_T \epsilon_A K_a)/(a_H + K_a)$ where $A_T$ is the total concentration of the compound, $a_H$ is the proton activity determined with a pH meter, $\epsilon_{HA}$ is the extinction coefficient of the acid species, $\epsilon_A$ is the extinction coefficient of the base species, and $K_a$ is the acid dissociation constant.

**Enzyme Assays.** Xanthine oxidase substrate studies were carried out as previously described.[3] PNPase activity (inosine to hypoxanthine and ribose-1-phosphate) was followed by measuring the increase in absorbance at 292 nm accompanying the oxidation of hypoxanthine to uric acid by xanthine oxidase.[20] The $\Delta\epsilon$ at 292 nm was found to be $1.29 \times 10^4$ M$^{-1}$ cm$^{-1}$, a value of $1.25 \times 10^4$ M$^{-1}$ cm$^{-1}$ was previously reported (at 293 nm).[24] Reaction mixtures contained 0.2 M pH 7.50 Tris buffer ($\mu$ = 1.0 KCl); 50 mM potassium phosphate; xanthine oxidase, 0.06 unit; PNPase, 0.003 unit; and inosine concentrations ranging from 0.020 to 0.50 mM. Stock solutions of inhibitors were prepared with dimethyl sulfoxide. The xanthine oxidase employed, grade III (Sigma), is chromatographically pure and reported to contain 36 units/mL. The unit activity of nucleoside phosphorylase from human blood (Sigma) was determined spectrophotometrically. Inosine and enzyme stock solutions were prepared in the same buffer solution used in the assay. All assays were carried out at 30 °C in a thermostated Perkin-Elmer 559 or λ3 spectrophotometer. The inhibitors 8-aminoquinazolin-4(3*H*)-one (**24**)[25] and 8-aminoquinazoline-2,4(1*H*,3*H*)-dione[26] were prepared as previously described.

Synthesis and physical properties of new compounds are provided below.

**4-Nitroimidazo[4,5-*g*]quinazolin-8(3*H*,7*H*)-one (3)** was previously prepared[3] in low yield. An improved synthesis is provided below.

To a solution of 3.5 mL of concentrated sulfuric acid and 3.5 mL of fuming nitric acid, chilled in an ice bath, was added 1.14 g (6.1 mmol) of imidazo[4,5-g]quinazolin-8(3*H*,7*H*)-one.[27] The reaction mixture was heated at 100 °C for 20 h and then poured over 50 g of cracked ice. The mixture was filtered immediately, and the filtrate was adjusted to pH 3 with KOH pellets. The nitro derivative crystallized from the liquor in pure form after sitting 3–4 h at room temperature: 1.1 g (78%) yield.

**4-Nitro-7-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)-imidazo[4,5-*g*]quinazolin-8(3*H*,7*H*)-one (6).** A suspension of

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2.0 g (8.6 mmol) of **3** in 160 mL of 1,1,1,3,3,3-hexamethyldisilazane (HMDS) and 16 mL of trimethylsilyl chloride was heated at reflux for 36 h. The solvents were then removed in vacuo, and the residue was dissolved in 100 mL of methylene chloride freshly distilled from $P_2O_5$. Imidazole ring desilylation was carried out by adding 350 μL of dry methanol to the methylene chloride solution and then stirring the mixture for 30 min. After desilylation, 2.7 g (8.6 mmol) of β-D-ribofuranose-1,2,3,5-tetra-*O*-acetate was added to the reaction mixture followed by addition of 8.6 mL of 1 M tin(IV) chloride in methylene chloride. The reaction was then stirred at room temperature for 30 min. All of the above steps were carried out under a blanket of dry nitrogen.

The completed reaction was diluted with 200 mL of chloroform and then with 200 mL of water. This mixture was shaken in a separatory funnel and the chloroform layer was separated. The aqueous layer was then extracted two more times with 200-mL portions of chloroform. Drying the combined chloroform extracts ($Na_2SO_4$) and then evaporation afforded a yellow oil, which largely consisted of the N(7)-nucleoside. The oil was placed on a silica gel (230–400 mesh) column (50 × 2.5 cm) prepared with chloroform. Elution with ethyl acetate afforded fractions containing the N(3)-nucleoside, which were immediately followed by fractions containing the N(7) nucleoside. The fractions were concentrated and the solids were recrystallized from ethyl acetate/hexane. Repeat experiments provided the following yields: 22–35% N(7)-nucleoside and a trace of the N(3)-nucleoside.

The N(7)-nucleoside was further purified by preparative silica gel TLC, employing ethyl acetate as the developing solvent: mp >110 °C dec; TLC (ethyl acetate) $R_f$ = 0.16; IR (thin film on KBr) 1748, 1690, 1616, 1534, 1374, 1235, 1100, 1050 $cm^{-1}$; $^1H$ NMR (dimethyl-$d_6$ sulfoxide) δ 8.74, 8.58 and 8.49 (3 H, 3 s, aromatic), 6.16 (1 H, d, $J$ = 4 Hz, C(1′)-H), 5.7 and 5.5 (2 H, 2 x m, C(2′)- and C(3′)-H, no assignments made), 4.3 (3 H, m, C(4′)- and C-(5′)-H), 2.10, 2.08, and 2.05 (9 H, 3 s, acetates); mass spectrum (EI mode), $m/z$ 489 ($P^+$). Anal. Calcd for $C_{20}H_{19}N_5O_{10}·0.5H_2O$: C, 48.19; H, 3.94; N, 14.04. Found: C, 48.41; H, 3.90; N, 13.33.

**4-Nitro-3-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)-imidazo[4,5-*g*]quinazolin-8(3*H*,7*H*)-one (7).** A solution of 1.63 g (7.0 mmol) of **3** in 130 mL of 1,1,1,3,3,3-hexamethyldisilazane and 13 mL of trimethylsilyl chloride was heated at reflux for 36 h. The solvents were then removed in vacuo, and the residue was dissolved in 100 mL of methylene chloride freshly distilled from $P_2O_5$. To the resulting solution was added 2.2 g (7.0 mmol) of β-D-ribofuranose-1,2,3,5-tetra-*O*-acetate followed by 7 mL of 1 M tin(IV) chloride in methylene chloride. The reaction mixture was then stirred for 30 min. All the steps described above were carried out under a blanket of dry nitrogen.

The completed reaction was diluted with 200 mL of chloroform and then with 200 mL of water. This mixture was shaken in a separatory funnel, and the chloroform layer was separated. The aqueous layer was then extracted two more times with 200-mL portions of chloroform. Drying the combined chloroform extracts ($Na_2SO_4$) and then evaporation afforded a yellow oil, which was largely the N(3)-nucleoside. Purification was carried out by flash chromatography (230–400-mesh silica gel with ethyl acetate as the eluant) followed by recrystallization from ethyl acetate/hexane: 1.9 g (55%) yeld; mp >105 °C dec; TLC (ethyl acetate) $R_f$ = 0.21; IR (thin film on KBr) 1750, 1695, 1621, 1539, 1371, 1225, 1110, 1059 $cm^{-1}$; $^1H$ NMR (dimethyl-$d_6$ sulfoxide) δ 8.91, 8.58, and 8.17 (3 H, 3 s, aromatic), 5.88 (1 H, d, $J$ = 4.9 Hz, C(1′)-H), 5.82 and 5.38 (2 H, m and t respectively, $J$ = 6 Hz for t, C(2′)- and C(3′)-H, no assignments made), 4.36 (1 H, m, C(4′)-H), 4.17 (2 H, m, C(5′)-H), 2.06 and 2.05 (6 H, 2 s, acetates), 1.82 (3 H, s, acetate); mass spectrum (EI mode), $m/z$ 489 ($P^+$). Anal. Calcd for $C_{20}H_{19}N_5O_{10}$: C, 49.08; H, 3.91; N, 14.30. Found: C, 48.93; H, 3.88; N, 12.67.

**4-Nitro-1-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)-imidazo[4,5-*g*]quinazolin-8(1*H*,7*H*)-one (8).** A suspension of 500 mg (2.16 mmol) of **3** in 50 mL of 1,1,1,3,3,3-hexamethyldisilazane and 5 mL of trimethylsilyl chloride was heated at reflux for 36 h. The reaction solvents were removed in vacuo, and the residue was dissolved in 50 mL of acetonitrile freshly distilled from $P_2O_5$. To this solution were added 690 mg (2.16 mmol) of β-D-ribofuranose-1,2,3,5-tetra-*O*-acetate and then 2.2 mL of 1 M tin(IV) chloride in methylene chloride. The reaction mixture was stirred for 15 h. All the steps described above were carried out under a blanket of dry nitrogen.

Chloroform extraction of the products was carried out as described for the preparation of the N(3) and N(7) isomers. Product purification was carried out by silica gel (230–400 mesh) chromatography on a 50 × 2.5 cm column employing ethyl acetate as the eluant. The order of elution of the nucleoside products and the yields obtained are as follows: **8**, 89.5 mg (8.5%); **7**, 77 mg (7.3%); and **6**, 201 mg (19%).

The N(1)-nucleoside was further purified by preparative silica gel TLC (ethyl acetate was the developing solvent) and recrystallization from ethyl acetate/hexane: mp >120 °C dec; TLC (ethyl acetate) $R_f$ = 0.25; IR (thin film on KBr) 1748, 1696, 1620, 1537, 1373, 1225, 1108, 1065 $cm^{-1}$; $^1H$ NMR (dimethyl-$d_6$ sulfoxide) δ 8.99, 8.75, and 8.19 (3 H, 3 s, aromatic), 6.60 (1 H, d, $J$ = 5.9 Hz, C(1′)-H), 5.69 and 5.46 (2 H, 2 t, $J$ = 6 Hz, C(2′)- and C(3′)-H, no assignments made), 4.52 (1 H, m, C(4′)-H), 4.4 (2 H, m, C-(5′)-H), 2.15 and 2.12 (6 H, 2 s, acetates), 2.05 (3 H, s, acetate); mass spectrum (EI mode), $m/z$ 489 ($P^+$). Anal. Calcd for $C_{20}H_{19}N_5O_{10}$: C, 49.08; H, 3.91; N, 14.30. Found: C, 48.93; H, 3.88; N, 12.67.

**4-Amino-7-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)-imidazo[4,5-*g*]quinazolin-8(3*H*,7*H*)-one (9).** To a solution of 192 mg (0.39 mmol) of **6** in 5 mL of methanol was added a solution of 300 mg of sodium dithionite in 2 mL of water. The reaction mixture was stirred for 30 s at room temperature and then combined with 50 mL of chloroform and 50 mL of water. The chloroform layer was separated, and the aqueous layer was extracted again with 50 mL of chloroform. The chloroform extracts were combined, dried ($Na_2SO_4$), and then concentrated in vacuo to a small volume. Addition of hexane of the concentrated solution resulted in precipitation of **9** as a crude yellow solid, 105-mg (58%) yield. Purification of the crude solid was carried out by flash chromatography on silica gel employing methanol-chloroform (10:90) as the eluant. The purified product was then recrystallized from ethyl acetate/hexane: TLC (methanol–chloroform [10:90]) $R_f$ = 0.14; mp 105–110 °C dec; IR (thin film on KBr) 1720, 1640, 1590, 1570, 1400, 1350, 1210, 1030, 740 $cm^{-1}$; $^1H$ NMR (dimethyl-$d_6$ sulfoxide) δ 8.33, 8.17, and 7.54 (3 H, 3 s, aromatic), 6.08 (1 H, d, $J$ = 4 Hz, C(1′)-H), 5.86 (2 H, br s, amine protons), 5.69 and 5.54 (2 H, 2 t, $J$ = 4 and 6 Hz, respectively, C(2′)- and C(3′)-H, no assignments made), 4.4–4.2 (3 H, m, C(4′)- and C(5′)-H), 2.10, 2.07, and 2.05 (9 H, 3 s, acetate methyls); mass spectrum (EI mode), $m/z$ 459 ($P^+$).

**4-Amino-3-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)-imidazo[4,5-*g*]quinazolin-8(3*H*,7*H*)-one (10).** To a solution of 127 mg (0.26 mmol) of **7** in 5 mL of methanol was added a solution of 200 mg of sodium dithionite in 2 mL of 0.2 M pH 8 phosphate buffer. After stirring the mixture for 1 min, 25 mL of chloroform was added to the mixture followed by 25 mL of water. The chloroform layer was separated, and the aqueous layer was extracted once more with 25 mL of chloroform. The chloroform extracts were dried ($Na_2SO_4$) and evaporated to a small volume. Addition of hexane afforded the crude amine, 63-mg (53%) yield. Purification of the crude solid was carried out by flash chromatography on silica gel, employing methanol–chloroform (10:90) as the eluant. The purified product was recrystallized from ethyl acetate/hexane: TLC (methanol–chloroform [10:90]) $R_f$ = 0.33; mp >85 °C dec; IR (thin film on KBr) 1720, 1630, 1580, 1405, 1340, 1210, 1030 $cm^{-1}$; $^1H$ NMR (dimethyl-$d_6$ sulfoxide) δ 8.59, 7.99, and 7.73 (3 H, 3 s, aromatic), 6.51 (1 H, d, $J$ = 6 Hz, C(1′)-H), 5.69 and 5.42 (2 H, 2 t, J = 6.5 Hz, C(2′)- and C(3′)-H, no assignments made), 5.52 (2 H, br s, amino), 4.46 (1 H, m, C(4′)-H), 4.36 (2 H, m, C(5′)-H), 2.11 (3 H, s, acetate), 2.04 (6 H, s, acetates); mass spectrum (EI mode), $m/z$ 459 ($P^+$).

**4-Amino-1-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)-imidazo[4,5-*g*]quinazolin-8(1*H*,7*H*)-one (11).** To a solution of 91.5 mg (0.18 mmol) of **8** in 5 mL of methanol was added a solution of 250 mg of sodium dithionite in 2 mL of water. After the reaction was stirred for 2 min, sodium dithionite (250 mg in 2 mL of water) was added again, and the reaction mixture was stirred for 2 more min. The reaction mixture was then diluted with 50 mL of chloroform and 50 mL of water, and the chloroform layer was separated after the mixture was vigorously shaken. The aqueous layer was extracted again with a 50-mL portion of chloforom. Evaporation of the dried extracts ($Na_2SO_4$) to a small volume and addition of hexane afforded **11** as an off-white solid:

35.6 mg (42%) yield; mp >120 °C dec; TLC (methanol–chloroform [10:90]) $R_f$ = 0.33; IR (thin film on KBr) 3335, 3275, 3200, 3125, 3065, 1748, 1670, 1601, 1384, 1228, 1107 cm$^{-1}$; $^1$H NMR (dimethyl-$d_6$ sulfoxide) δ 8.50, 7.86, and 7.56 (3 H, 3 s, aromatic), 6.29 (1 H, d, $J$ = 6.5 Hz, C(1′)-H), 5.88 (2 H, br s, amino), 5.63 and 5.37 (2 H, 2 t, $J$ = 5.9 and 5 Hz, C(2′)- and C(3′)-H, no assignments made), 4.4 (1 H, m, C(4′)-H), 4.32 (2 H, br s, C(5′)-H), 2.09, 2.08, and 1.95 (9 H, 3 s, acetate methyls); mass spectrum (EI mode), $m/z$ 459 (P$^+$).

**7-(2′,3′,5′-Tri-*O*-acetyl-β-D-ribofuranosyl)imidazo[4,5-*g*]quinazoline-4,8,9(3*H*,7*H*)-trione (12).** To a solution of 187 mg (0.4 mmol) of **9** in 3 mL of dimethylformamide was added a solution of 868 mg (3.2 mmol) of Fremy salt in 40 mL of water. The mixture was stirred for 1.5 h and then extracted with 3 × 100-mL portions of chloforom. The dried ($Na_2SO_4$) chloroform extracts were concentrated to an oil, which was triturated with diethyl ether to afford the crude quinone as a yellow solid (158 mg, 83% yield). Purification for both characterization and the deacetylation step was carried out by preparative chromatography on silica gel employing 10% methanol in chloroform as the eluant: mp 147–150 °C dec; TLC (10% methanol in chloroform) $R_f$ = 0.15; IR (KBr pellet) 1745, 1715, 1700, 1505, 1365, 1235, 1055 cm$^{-1}$; $^1$H NMR (dimethyl-$d_6$ sulfoxide) δ 8.99 and 8.21 (2 H, 2 s, aromatic), 6.04 (1 H, d, $J$ = 2.8 Hz, C(1′)-H), 5.63 and 5.52 (2 H, 2 m, C(2′)- and C(3′)-H), no assignments made), 4.4–4.2 (3 H, m, C(4′)- and C(5′)-H), 2.11 (3 H, s, acetate), 2.069 and 2.064 (6 H, 2 s, acetates); mass spectrum (EI mode, solids probe), $m/z$ 476 (P$^+$ + 2), 474 (P$^+$). The P$^+$ + 2 mass corresponds to hydroquinone formed on solids probe. Anal. Calcd for $C_{20}H_{18}N_4O_{10}\cdot H_2O$: C, 48.78; H, 4.09; N, 11.37. Found: C, 48.83; H, 3.65; N, 10.94.

**3-(2′,3′,5′-Tri-*O*-acetyl-β-D-ribofuranosyl)imidazo[4,5-*g*]quinazoline-4,8,9(3*H*,7*H*)-trione (13).** To a solution of 434 mg (0.94 mmol) of **10** in 5 mL of dimethylformamide was added 2.5 g (9.3 mmol) of Fremy's salt in 75 mL of water. The reaction was stirred at room temperature for 4 h and then extracted with 3 × 100-mL portions of chloroform. The dried extracts ($Na_2SO_4$) were concentrated to an oil, which was diluted with 50 mL of diethyl ether and 10 mL of hexane. The precipitate was filtered, washed with hexane, and dissolved in a small volume of ethyl acetate. This solution afforded **13** as a crystalline solid after sitting at room temperature for 4 h, 127-mg (28%) yield. An analytically pure sample was obtained by recrystallization from hot methanol: mp >190 °C dec; TLC (1-butanol–acetic acid–water [5:2:3]) $R_f$ = 0.43; TLC (methanol–chloroform [20:80]) $R_f$ = 0.08; IR (thin film on KBr) 1745, 1640, 1550, 1475, 1370, 1224, 1085 cm$^{-1}$; $^1$H NMR (dimethyl-$d_6$ sulfoxide) δ 8.52 and 8.49 (2 H, 2 s, aromatic), 6.44 (1 H, d, $J$ = 4.3 Hz, C(1′)-H), 5.59 and 5.42 (2 H, 2 t, $J$ = 6 Hz, C(2′) and C(3′)-H, no assignments made), 4.4–4.3 (3 H, m, C(4′)- and C(5′)-H), 2.056 (6 H, s, acetates), 2.012 (3 H, s, acetate); mass spectrum (EI mode, solids probe) $m/z$ 476 (P$^+$ + 2, hydroquinone). Anal. Calcd for $C_{20}H_{18}N_4O_{10}$: C, 50.64; H, 3.82; N, 11.80. Found: C, 51.17; H, 3.17, H, 11.46.

**1-(2′,3′,5′-Tri-*O*-acetyl-β-D-ribofuranosyl)imidazo[4,5-*g*]quinazoline-4,8,9(1*H*,7*H*)-trione (14).** To a solution of 35 mg (0.07 mmol) of **11** in 0.5 mL of dimethylformamide was added 150 mg (0.56 mmol) of Fremy's salt in 10 mL of water containing 200 mg of monobasic potassium phosphate. After stirring for 15 h at room temperature, the reaction was extracted with 3 × 20-mL portions of chloroform. The dried extracts ($Na_2SO_4$) were concentrated to an oil, from which the crude quinone was precipitated by addition of diethyl ether/hexane. Purification was carried out by preparative TLC, employing 20% methanol in chloroform as the eluant: ~5-mg (15%) yield; mp 150 °C dec; TLC (1-butanol–acetic acid–water [5:2:3]) $R_f$ = 0.51; TLC (methanol–chloroform [20:80]) $R_f$ = 0.12; IR (thin film on KBr) 1751, 1700, 1653, 1470, 1380, 1229, 1060 cm$^{-1}$; $^1$H NMR (dimethyl-$d_6$ sulfoxide) δ 8.44 and 8.43 (2 H, 2 s, aromatic), 6.56 (1 H, d, $J$ = 4.4 Hz, C(1′)-H), 5.55 and 5.40 (2 H, 2 m, C(1′)- and C(2′)-H, no assignments made), 4.35 (3 H, m, C(4′)- and C(5′)-H), 2.05 (6 H, s, acetates), 2.03 (3 H, s, acetate); mass spectrum (solids probe), $m/z$ 476 (P$^+$ + 2, hydroquinone).

**7-β-D-Ribofuranosylimidazo[4,5-*g*]quinazoline-4,8,9-(3*H*,7*H*)-trione (15).** To a solution of 187 mg (0.38 mmoles) of **12** in 5 mL of dry methanol was added 1 mL of 5% methanolic NaOH. The reaction was stirred for 10 min, during which time the red sodium salt of the quinone precipitated from solution. Acidification of the reaction with acetic acid and then dilution with 5 mL of water resulted in crystallization of the deblocked nucleoside **15**. The product was filtered, washed with water, and dried: 66-mg (46%) yield; TLC (1-butanol–water–acetonitrile [5:2:3]) $R_f$ = 0.3; IR (KBr pellet), 3400, 1700, 1684, 1640, 1576, 1501, 1398, 1108, 1063, 899 cm$^{-1}$; $^1$H NMR (dimethyl-$d_6$ sulfoxide) δ 9.20 and 8.09 (2 H, 2 s, aromatic), 5.89 (1 H, s, C(1′)-H), 5.6, 5.3, and 5.06 (3 H, 3 br s, ribofuranosyl hydroxyls), 4.05 and 3.93 (3 H, 2 × m, C(2′)-, C(3′)-, and C(4′)-H), 3.80 and 3.60 (2 H, 2 m, C(5′)-H). Anal. Calcd for $C_{14}H_{12}N_4O_7\cdot 1.5H_2O$: C, 44.80; H, 4.02; N, 14.92. Found: C, 44.53; H, 3.72; N, 14.57.

**3-β-D-Ribofuranosylimidazo[4,5-*g*]quinazoline-4,8,9-(3*H*,7*H*)-trione (1).** Deacetylation of **13** was carried out using the procedure for deacetylation of the N(7) isomer **12**. The N(3) isomer **1** crystallized from the reaction mixture as yellow needles: 88% yield; TLC (1-butanol–acetic acid–water [5:2:3]), $R_f$ = 0.31; IR (KBr pellet) 3350, 1695, 1619, 1478, 1261, 1101, 1054, 1026, 808 cm$^{-1}$; $^1$H NMR (dimethyl-$d_6$ sulfoxide) δ 8.70 and 8.53 (2 H, 2 s, aromatic), 6.22 (1 H, d, $J$ = 3.2, C(1′)-H), 5.56 (1 H, d, $J$ = 5.8 Hz, hydroxyl), 5.16 (2 H, m, hydroxyls), 4.17, 4.08, and 3.94 (3 H, 3 m, C(2′)-, C(3′)-, and C(4′)-H, no assignments made), 3.72 and 3.59 (2 H, 2 m, C(5′)-H). Anal. Calcd for $C_{14}H_{12}N_4O_7\cdot 0.5H_2O$: C, 47.06; H, 3.66; N, 15.67. Found: C, 47.04; H, 3.63; N, 15.64.

**1-β-D-Ribofuranosylimidazo[4,5-*g*]quinazoline-4,8,9-(1*H*,7*H*)-trione (16).** Deacetylation of **14** was carried out using the procedure for deacetylation of the N(7) isomer **12**. The N(1) isomer **16** precipitated from the reaction mixture as an amorphous solid: 47% yield; TLC (1-butanol–acetic acid–water [5:2:3]) $R_f$ = 0.31; $^1$H NMR (dimethyl-$d_6$ sulfoxide) δ 8.68 and 8.50 (2 H, 2 s, aromatic protons), 6.26 (1 H, d, $J$ = 2.7 Hz, C(1′)-H), 5.61 and 5.10 (2 H, 2 d, $J$ = 5.0 Hz, $J$ = 5.6 Hz, 2′- and 3′-hydroxyls, no assignments made), 5.19 (1 H, t, $J$ = 4.8 Hz, 5′-hydroxyl), 4.09 (2 H, m, C(2′)- and C(3′)-H, no assignments made), 3.9 (1 H, m, C(4′)-H), 3.7 and 3.6 (2 H, 2 m, C(5-)-H). Anal. Calcd for $C_{14}H_{12}N_4O_7\cdot 2H_2O$: C, 47.45; H, 5.12; N, 15.80. Found: C, 47.51; H, 5.05; N, 15.83.

**7-Methylimidazo[4,5-*g*]quinazoline-4,8,9(3*H*,7*H*)-trione (19H)** was prepared as outlined below.

A suspension of 3.0 g (14.5 mmol) of 5-nitrobenzimidazole-6-carboxylic acid in thionyl chloride (10.0 mL) was refluxed for 3.0 h. The excess thionyl chloride was evaporated in vacuo, and the residue was suspended in 4 mL of dry dimethylformamide (DMF). To this solution was added 30 mL of 17% methylamine in DMF, and the mixture was stirred at 25 °C for 10 min. The DMF was evaporated in vacuo and the residue was suspended in 40 mL of water. The aqueous mixture was extracted with 10 × 100-mL portions of ethyl acetate. The extracts were then dried ($Na_2SO_4$) and concentrated in vacuo to dryness. Recrystallization of the solid from ethyl acetate–hexane afforded **6-(*N*-methylcarbamyl)-5-nitrobenzimidazole**: 1.12-g (35%) yield; mp >250 °C; TLC (2-propoanol–water–ammonium hydroxide [7:2:1]) $R_f$ = 0.75; IR (KBr) 3314,3183, 1650, 1577, 1532, 1326, 1285, 1111, 934, 752 cm$^{-1}$; $^1$H NMR (dimethyl-$d_6$ sulfoxide) δ 8.54, 8.26, and 7.70 (3 H, 3 s, aromatic protons), 8.49 (1 H, br m, amide proton), 2.73 (3 H, d, $J$ = 4.6 Hz, N(7)-methyl). Addition of $D_2O$ resulted in collapse of the N(7)-methyl doublet to a singlet.

A mixture of 900 mg (4.1 mmol) of 6-(*N*-methylcarbamyl)-5-nitrobenzimidazole and 200 mg of 5% Pd/C in 50 mL of methanol was shaken under 50 psi of $H_2$ for 5 h. The completed reaction was filtered through Celite and evaporated in vacuo to a solid. The solid was dissolved in 30 mL of 96% formic acid and refluxed for 4 h, after which the solution was evaporated in vacuo to afford a gray solid. Recrystallization from ethanol–hexane afforded 572 mg (70%) yield of **7-methylimidazo[4,5-*g*]quinaozlin-8-(3*H*,7*H*)-one.** An analytical sample was prepared by a second recrystallization from ethanol–hexane: mp 115–120 °C; TLC (butanol–acetic acid–water [5:2:3]), $R_f$ = 0.53; IR (KBr) 3177, 3162, 1668, 1617, 1339, 1293, 1272, 1048, 846, 786 cm$^{-1}$; $^1$H NMR (dimethyl-$d_6$ sulfoxide) δ 8.48, 8.33, 8.23, and 7.78 (4 H, 4 s, aromatic protons), 3.47 (3 H, s, N(7)-methyl); mass spectrum (EI mode), $m/z$ 200 (P$^+$).

A solution of 200 mg (1 mmol) of 7-methylimidazo[4,5-*g*]-quinazolin-8(3*H*,7*H*)-one in 6 mL of concentrated sulfuric acid and 6 mL of 90% nitric acid was heated at 120 °C for 20 h. The reaction mixture was poured over 200 g of ice, and the pH of the resulting solution adjusted to 6 with ammonium hydroxide/acetic

acid. The product was extracted with 10 × 25-mL portions of ethyl acetate. Concentration of the dried ($Na_2SO_4$) extracts and recrystallization of the residue from methanol afforded **7-methyl-4-nitroimidazo[4,5-*g*]quinazolin-8(3*H*,7*H*)-one** as yellow needles: 88 mg (36% yield); mp 345–350 °C dec; TLC (chloroform–methanol [90:10]) $R_f$ = 0.13; IR (KBr pellet) 2950, 1650, 1560, 1480, 1280, 1220 $cm^{-1}$; $^1H$ NMR (dimethyl-$d_6$ sulfoxide) δ 8.64, 8.50, and 8.37 (3 H, 3 s, aromatic protons), 3.49 (3 H, s, N(7)-methyl).

A mixture of 80 mg (0.36 mmol) of 7-methyl-4-nitroimidazo-[4,5-*g*]quinazolin-8(3*H*,7*H*)-one and 30 mg of 5% Pd/C in 50 mL of methanol was shaken under 40 psi of $H_2$ for 3 h. The completed reaction was filtered through Celite, and the filtrate was evaporated to dryness. The residue was dissolved in 10 mL of dimethylformamide and mixed with a solution consisting of 500 mg of potassium monophosphate and 500 mg of Fremy's salt in 30 mL of water. The reaction mixture was then stirred at 25 °C for 12 h. The yellow solid that formed was collected by vacuum filtration and recrystallized from hot water to afford **19H**: 47 mg (57%) yield; TLC (butanol–water–acetic acid [5:2:3]) $R_f$ = 0.3; IR (KBr pellet) 3517, 2925, 1693, 1580, 1497, 1392, 1027, 996, 903, 773, 606 $cm^{-1}$; $^1H$ NMR (dimethyl-$d_6$ sulfoxide) δ 8.86 and 8.21 (2 H, 2 s, aromatic protons), 3.46 (3 H, s, N(7)-methyl); mass spectrum (EI mode, solids probe), *m/z* 232 ($P^+$ + 2), 230 ($P^+$). The $P^+$ + 2 mass corresponds to hydroquinone formed on solids probe.

**8-Nitro-3-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)-quinazolin-4(3*H*)-one (21).** A solution of 500 mg (0.52 mmol) of **20**[25] in 40 mL of 1,1,1,3,3,3-hexamethyldisilazane and 2.0 mL of trimethylsilyl chloride was heated at reflux for 36 h. The solvents were then removed in vacuo, and the residue was dissolved in 60 mL of acetonitrile freshly distilled from $P_2O_5$. To the resulting solution was added 1.0 g of β-D-ribofuranose-1,2,3,5-tetra-*O*-acetate followed by 3.0 mL of 1 M tin(IV) chloride in methylene chloride. The reaction mixture was then stirred for 2 h. All the steps described above were carried out under a blanket of dry nitrogen.

The completed reaction was diluted with 200 mL of chloroform and then with 200 mL of water. This mixture was shaken in a separatory funnel, and the chloroform layer was separated. The aqueous layer was then extracted twice with 200-mL portions of chloroform. The combined chloroform extracts were dried ($Na_2SO_4$) and then evaporated to a homogeneous oil, which was a mixture of product, **20**, and unreacted sugar. Purification was carried out by column chromatography (230–400-mesh silica gel with 3% methanol in chloroform as eluant) followed by precipitation from carbon tetrachloride–hexane: 526-mg (45%) yield; mp 57–62 °C dec; TLC (chloroform–acetone [9:1]) $R_f$ = 0.41; IR (KBr) 1751, 1696, 1615, 1536, 1373, 1231, 1098, 1065, 773 $cm^{-1}$; $^1H$ NMR (dimethyl-$d_6$ sulfoxide) δ 8.57 (1 H, s, C(2)-H), 8.41 and 8.36 (2 H, 2 d, *J* = 8.0 Hz, C(5)- and C(7)-H, no assignments made), 7.74 (1 H, t, *J* = 8.0 Hz, C(6)-H), 6.13 (1 H, d, *J* = 3.9 Hz, C(1′)-H), 5.68 and 5.52 (2 H, dd and t, *J* = 3.9 and 6.3 Hz for dd, *J* = 6.4 Hz for t, C(2′)- and C(3′)-H, no assignments made), 4.39 and 4.28 (3 H, 2 m, C(4′)-H and C(5′)-H, no assignemnts made), 2.08 and 2.04 (9 H, 2 s, acetates, no assignments made); mass spectrum (EI mode), *m/z* 449 ($P^+$). Anal. Calcd for $C_{19}H_{19}N_3O_{10}·0.6H_2O$: C, 49.58; H, 4.39; N, 9.13. Found: C, 49.34; H, 4.12; N, 8.92.

**8-Amino-3-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)-quinazolin-4(3*H*)-one (22).** A mixture of **21** (100 mg, 0.22 mmol) and 5% Pd/C (40 mg) in methanol (40 mL) was shaken under 30 psi of $H_2$ for 5.0 min. The completed reaction was filtered through Celite and the filtrate evaporated to a green oil. Purification was carried out by flash chromatography (230–400-mesh silica gel with 10% acetone in chloroform as eluant) followed by recrystallization from ether–hexane: 56-mg (60%) yeild; mp 55–60 °C dec; TLC (ethyl acetate) $R_f$ = 0.51; IR (KBr) 3592, 3378, 1749, 1682, 1618, 1601, 1374, 1230, 1096, 1052, 761 $cm^{-1}$; $^1H$ NMR (chloroform-$d_1$–dimethyl-$d_6$ sulfoxide [2:1]) δ 8.20 (1 H, s, C(2)-H), 7.37 (1 H, d, *J* = 8.0 Hz, C(5)-H), 7.22 (1 H, t, *J* = 8.0 Hz, C(6)-H), 7.05 (1 H, d, *J* = 8.0 Hz, C(7)-H), 6.07 (1 H, d, *J* = 4.0, C(1′)-H), 5.64 and 5.54 (2 H, dd and t, *J* = 4.0 and 6.2 Hz for dd, *J* = 6.2 Hz for t, C(2′)- and C(3′)-H, no assignments made), 5.41 (2 H, br s, C(8)-amino), 4.44 and 4.31 (2 H, 2 dd, *J* = 2.9 and 11.7 Hz, *J* = 5.1 and 11.7 Hz, C(5′)-H), 4.37 (1 H, m, C(4′)-H), 2.11, 2.10, and 2.09 (9 H, 3 s, acetates, no assignments made). Addition of $D_2O$ resulted in the collapse of the C(8)-amino protons. The assignments of the aromatic protons are based on NOEs with the C(8)-amino protons.

**4-[*N*-(2′,3′,5′-Tri-*O*-acetyl-β-D-ribofuranosyl)-carbamyl]-1,2,3-benzotriazole (23).** A mixture of **21** (250 mg, 0.56 mmol) and 5% Pd/C (100 mg) in 35 mL of dimethylformamide was shaken under 30 psi of $H_2$ for 30 min. The completed reaction was filtered through Celite into a flask containing a solution of Fremy's salt (2.0 g, 7.2 mmol) dissolved in 35 mL of water. The resulting solution was stirred for 1.0 h at 25 °C. The completed reaction was diluted by the addition of ethyl acetate (100 mL) and water (50 mL) and then shaken in a separatory flask. The organic phase was washed with water (4 × 50 mL), dried over $Na_2SO_4$, and evaporated to a homogeneous oil. Purification was carried out by flash chromatography (200–400-mesh silica gel with 20% hexane in ethyl acetate as eluant) followed by precipitation from chloroform–hexane: 105-mg (45%) yield; mp 178–180 °C dec; TLC (chloroform–methanol [9:1]) $R_f$ = 0.58; IR (KBr) 3082, 1748, 1674, 1662, 1613, 1561, 1378, 1286, 1040 $cm^{-1}$; $^1H$ NMR (dimethyl-$d_6$ sulfoxide) δ 9.63 (1 H, br s, amide), 8.20 and 8.04 (2 H, br d and d, *J* = 7.8 Hz, *J* = 7.4 Hz, respectively, C(5)- and C(7)-H, no assignments made), 7.52 (1 H, t, *J* = 7.9 Hz, C(6)-H), 5.79 (1 H, dd, *J* = 3.8 and 8.6 Hz, C(1′)-H), 5.34 (2 H, m, C(2′)- and C(3′)-H), 4.25 and 4.09 (2 H, 2 dd, *J* = 3.5 and 11.3 Hz, *J* = 5.0 and 11.3 Hz, C(5′)-H), 4.15 (1 H, m C(4′)-H), 2.04, 2.03, and 2.01 (9 H, 3 s, acetates). Addition of $D_2O$ resulted in collapse of the C(1′)-proton to a doublet (*J* = 4.0 Hz). Coupling between the amide and C(1′)-protons was further established by COSY. Mass spectrum (EI mode) *m/z* 419 ($P^+$ − 1). Anal. Calcd for $C_{18}H_{20}N_4O_8$: C, 51.43; H, 4.76; N, 13.33. Found: C, 51.60; H, 4.77; N, 13.12.

**4-[*N*-(β-D-Ribofuranosyl)carbamyl]-1,2,3-benzotriazole (Deacetylated 23).** A solution of 50 mg (0.12 mmol) of **23** in 0.2 M methanolic sodium hydroxide solution (2.0 mL) was stirred at 25 °C for 30 min. The completed reaction was evaporated to dryness, and the residue was dissolved in 1.0 mL of pH 5 acetate buffer (0.2 M). The solution was adjusted to pH 6 by addition of acetic acid and chromatographed through a desalting column (27 × 3 cm, Biorad Bio-gel P-2, eluted with water). The nucleoside fractions were combined and evaporated to afford a homogeneous oil, which was precipitated from methanol–chloroform: 23-mg (66%) yield; mp >125 °C dec; TLC (butanol–acetic acid–water [5:3:2]) $R_f$ = 0.6; IR (KBr) 3413, 1653, 1599, 1544, 1381, 1108, 1046 $cm^{-1}$; $^1H$ NMR (dimethyl-$d_6$ sulfoxide) δ 10.14 (1 H, d, *J* = 9.0 Hz, amide), 7.96 and 7.78 (2 H, 2 d, *J* = 7.9 Hz, *J* = 6.7 Hz, C(5)-H and C(7)-H, no assignments made), 7.19 (1 , t, *J* = 7.6 Hz, C(6)-H), 5.54 (1 H, dd, *J* = 4.5 and 9.0 Hz, C(1′)-H), 5.1 (3 H, br s, hydroxides), 3.94 (2 H, m, C(2′)- and C(3′)-H), 3.72 (1 H, m, C(4′)-H), 3.51 and 3.43 (2 H, 2 dd, *J* = 4.1 and 11.6 Hz, *J* = 5.1 and 11.6 Hz, C(5′) diastereomeric protons coupled to each other, 11.6 Hz, and coupled to the C(4′) proton, 4.1 and 5.1 Hz). Addition of $D_2O$ resulted in collapse of the C(1′)-proton to a doublet (*J* = 4.4 Hz). Coupling between the amide and C(1′)-protons was further established by COSY: quantitative $^{13}C$ NMR (dimethyl-$d_6$ sulfoxide) δ 165 (1 C, amide carbonyl), 144, 140, 123, 121, 120, and 119 (6 C, aromatic carbons), 84 (2 C, ribofuranosyl carbons), 74, 70, and 62 (3 C, ribofuranosyl carbons); mass spectrum (EI mode) *m/z* 294 ($P^+$).

**5-Aminoimidazo[4,5-*g*]quinazolin-8(3*H*,7*H*)-one (25).** A solution of sodium dithionite (2.5 g) in 10 mL of water was added dropwise over a 30-min period to a suspension of **3** in 12 mL of dimethyl sulfoxide stirred at 100 °C. The reaction's progress was monitored by TLC (butanol–acetic acid–water [5:3:2]). The completed reaction was cooled to 25 °C, diluted with 50 mL of water, and adjusted to pH 7 by addition of solid sodium bicarbonate. The tan solid was collected, rinsed with generous portions of water and ethanol, and then dried in vacuo: 521-mg (80%) yield; mp >250 °C; TLC (butanol–acetic acid–water [5:3:2]) $R_f$ = 0.56; IR (KBr) 3351, 3046, 2774, 1642, 1610, 1430, 1119, 906 $cm^{-1}$; $^1H$ NMR (dimethyl-$d_6$ sulfoxide) δ 8.24, 7.81, and 7.41 (3 H, 3 s, aromatic), 5.70 (2 H, br s, C(4)-amino); mass spectrum (EI mode), *m/z* 201 ($P^+$). Anal. Calcd for $C_9H_7N_5O·0.2H_2O$: C, 52.79; H, 3.62; N, 34.21. Found: C, 53.03; H, 3.65; N, 33.79.

**8-[(2′,3′,5′-Tri-*O*-benzoyl-β-D-ribofuranosyl)amino]-quinazolin-4(3*H*)-one (Benzoylated 2).** To a mixture of 500 mg (3.1 mmol) of **24**,[25] 1.60 g (3.2 mmol) of 1-*O*-acetyl-2,3,5-tri-

O-benzoyl-β-D-ribofuranose, and 200 mg (1.4 mmol) of flame-dried potassium carbonate in 25 mL of freshly-distilled acetonitrile was added 1.2 mL (6.3 mmol) of trimethylsilyl trifluoromethanesulfonate under a nitrogen atmosphere. The clear solution was stirred at 25 °C for 8 h, during which time 50-mg portions of potassium carbonate were added when the previous portion dissolved. The crystals were filtered off, rinsed with a small portion of acetonitrile and then with generous portions of water, and dried in vacuo: 952-mg (51%) yield; mp 200-202 °C dec; TLC (ethyl acetate) $R_f$ = 0.5; IR (KBr pellet) 3414, 3265, 1717, 1582, 1504, 1452, 1273, 1133, 709 cm$^{-1}$; $^1$H NMR (dimethyl-$d_6$ sulfoxide) δ 9.29 (1 H, br s, N(3)-H), 8.21–6.88 (20 H, m, aromatic protons, C(2)-H, and 8-amino), 6.03 (1 H, dd, $J$ = 5.7 and 9.7 Hz, C(1′)-H), 5.91 (1 H, dd, $J$ = 3.2 and 5.7 Hz, C(3′)-H), 5.73 (1 H, t, $J$ = 5.7 Hz, C(2′)-H), 4.55 (3 H, m, C(4′)- and C(5′)-H). Addition of $D_2O$ resulted in collapse of the C(1′)-H quartet to a doublet ($J$ = 5.4 Hz). Ribofuranosyl proton assignments were established by COSY: mass spectrum (EI mode), $m/z$ 605 (P$^+$). Anal. Calcd for $C_{34}H_{27}N_3O_8$: C, 67.44; H, 4.46; N, 6.94. Found: C, 67.67; H, 4.54; N, 6.71.

**8-(β-D-Ribofuranosylamino)quinazolin-4(3*H*)-one Sodium Salt (2).** A solution of benzoylated **2** in 5 mL of 0.18 M methanolic sodium hydroxide was stirred at 25 °C for 30 min. The crystals that formed were filtered off, rinsed with methanol, and dried: 93-mg (89%) yield; mp 197–199 °C dec; TLC (butanol–ethanol–water [80:10:25]) $R_f$ = 0.5; IR (KBr pellet) 3397, 3213, 1516, 1443, 1334, 1119, 1002, 758 cm$^{-1}$; $^1$H NMR (dimethyl-$d_6$ sulfoxide) δ 8.04 (1 H, s, C(2)-H), 7.22 and 6.69 (2 H, 2 d, $J$ = 7.8 Hz, $J$ = 7.5 Hz, C(5)-H and C(7)-H, no assignments made), 7.05 (1 H, d, $J$ = 9.5 Hz, C(8)-amino), 5.38 and 5.08 (3 H, 2 br s, C(2′)-, C(3′)-, and C(5′)-OH, no assignments made), 4.88 (1 H, dd, $J$ = 1.9 and 9.6 Hz, C(1′)-H), 3.73 (2 H, m, C(2′)- and C(3′)-H), 3.64 and 3.37 (3 H, 2 m, C(4′)- and C(5′)-H, no assignments made). Collapse of the C(1′) quartet to a closely spaced doublet resulted from addition of $D_2O$, and from homonuclear decoupling of the C-(8)-amino proton: mass spectrum (EI mode), $m/z$ 293 (P$^+$). Anal. Calcd for $C_{13}H_{14}N_3O_5Na\cdot1.3H_2O$: C, 46.10; H, 4.91; N, 12.41. Found: C, 46.27; H, 4.87; N, 12.19.

The acid form was obtained by dissolving 100 mg (0.32 mmol) of the sodium salt in 0.2 M pH 7.0 potassium phosphate buffer (2.0 mL). The crystals that formed were collected and rinsed sparingly with cold water: 80 mg (86%) yield.

**4-[(2′,3′,5′-Tri-*O*-benzoyl-β-D-ribofuranosyl)amino]-imidazo[4,5-*g*]quinazolin-8(3*H*,7*H*)-one (Benzoylated 26).** To a mixture of 200 mg (1.0 mmol) of **25** and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (510 mg, 1.0 mmol) in 35 mL of freshly distilled acetonitrile was added 1.2 mL (6.3 mmol) of trimethylsilyl trifluoromethanesulfonate under a nitrogen atmosphere. The clear solution was stirred at 25 °C for 45 min and then diluted with 100 mL of ethyl acetate followed by 50 mL of 5% aqueous sodium bicarbonate solution. This mixture was shaken in a separatory funnel, and the ethyl acetate layer was separated and washed with 50 mL of water. The ethyl acetate extract was dried ($Na_2SO_4$) and then evaporated to homogeneous oil, which consisted of product and unreacted starting materials. Purification was carried out by flash chromatography (230–400-mesh silica gel with 7% methanol in chloroform as eluant) followed by recrystallization from ethyl acetate–hexane: 241-mg (38%) yield; mp 133–135 °C dec; TLC (ethyl acetate–methanol [9:1]) $R_f$ = 0.51; IR (KBr) 3349, 1727, 1668, 1584, 1515, 1452, 1271, 1119, 1069, 710 cm$^{-1}$; $^1$H NMR (dimethyl-$d_6$ sulfoxide) δ 8.37–7.09 (19 H, m, C(5)-amino and aromatic protons), 5.95 (1 H, dd, $J$ = 3.5 and 5.7 Hz, C(1′)-H), 5.83 (2 H, m, C(2′)- and C(3′)-H), 4.62 and 4.50 (3 H, 2 m, C(4′)- and C(5′)-H, no assignments made). Addition of $D_2O$ resulted in collapse of the C(1′) quartet to a doublet ($J$ = 3.5 Hz). Anal. Calcd for $C_{35}H_{27}N_5O_8\cdot0.5H_2O$: C, 64.22; H, 4.28; N, 10.70. Found: C, 64.30: H, 4.08; N, 10.42.

**2,8-Diaminoquinazolin-4(3*H*)-one (2-amino-24)** was prepared in three steps: A solution consisting of 2.0 g (9.3 mmol) of 2-amino-5-bromobenzoic acid, 600 mg (14.3 mmol) of cyanamide, and 0.4 mL of concentrated HCl in 30 mL of absolute ethanol was stirred at reflux for 6 h. At 1-h intervals, 0.2 mL of concentrated HCl was added to the reaction mixture. The crystals that formed were filtered and rinsed with ethanol: 889-mg (40%) yield of 2-amino-6-bromoquinazolin-3(4*H*)-one; mp >250 °C dec; TLC (ethyl acetate–acetic acid [9:1]) $R_f$ = 0.5; IR (KBr pellet) 3311, 3163, 1720, 1694, 1657, 1618, 1469, 1342, 826, 638 cm$^{-1}$; $^1$H NMR (dimethyl-$d_6$ sulfoxide) δ 7.96 (1 H, d, $J$ = 2.4 Hz, C(5)-H), 7.77 (1 H, dd, $J$ = 2.4 and 8.8 Hz, C(7)-H), 7.42 (2 H, br s, C(2)-amino), 7.25 (1 H, d, $J$ = 8.8 Hz, C(8)-H). Addition of $D_2O$ resulted in the collapse of the C(2)-amino protons.

The 2-amino-6-bromoquinazolin-3(4*H*)-one (5.0 g, 20.9 mmol) was added to an ice-cooled solution of 120 mL of red fuming nitric acid. The reaction was heated to 80 °C and stirred for 2.0 h. Addition of 125 g of cracked ice afforded 2-amino-6-bromo-8-nitroquinazolin-4(3*H*)-one as a yellow precipitate. Recrystallization was carried out from boiling water: 3.2-g (54%) yield; mp >200 °C dec; TLC (ethyl acetate–acetic acid [9:1]) $R_f$ = 0.6; IR (KBr) 3326, 3075, 1658, 1630, 1530, 1458, 800, 755 cm$^{-1}$; $^1$H NMR (dimethyl-$d_6$ sulfoxide) δ 8.27 and 8.09 (2 H, 2 d, $J$ = 2.4 Hz, C(5)-H and C(7)-H, no assignments made), 6.95 (2 H, br s, C-(2)-amino). Addition of $D_2O$ resulted in collapse of the C(2)-amino protons; mass spectrum (EI mode), $m/z$ 284 (P$^+$, $^{79}$Br), 286 (P$^+$, $^{81}$Br).

A solution of 1.0 g (3.5 mmol) of 2-amino-6-bromo-8-nitroquinazolin-4(3*H*)-one in 100 mL of methanol, containing 300 mg of 5% Pd on charcoal, was reduced under 50 psi of $H_2$ for 24 h. The catalyst was removed by filtration through Celite, and the filtrate was acidified by addition of 2 mL of concentrated HCl. The solvent was evaporated in vacuo, and the solid residue was recrystallized from methanol to afford the amine hydrochloride as a brown solid: 512-mg (69%) yield; mp >250 °C dec; TLC (butanol–acetic acid–water [5:3:2]) $R_f$ = 0.6; IR (KBr pellet) 3047, 2916, 2862, 2810, 1733, 1695, 1610, 1501, 1318, 759, 512 cm$^{-1}$; $^1$H NMR (dimethyl-$d_6$ sulfoxide) δ 7.84 (2 H, br s, amino, no assignment made), 7.30 and 7.09 (1 H and 2 H, d and m, $J$ = 8.2 Hz for d, aromatic protons, no assignments made); addition of $D_2O$ resulted in collapse of the amino protons; mass spectrum (EI mode) $m/z$ 176 (P$^+$). Anal. Calcd for $C_8H_8N_4O\cdot2HCl$: C, 38.57; H, 4.05; N, 22.50. Found: C, 38.95; H, 4.02; N, 21.98.

The hydrochloride salt was converted to the free amine by recrystallization from hot 0.2 M pH 7.5 potassium phosphate buffer: $^1$H NMR (dimethyl-$d_6$ sulfoxide) δ 10.79 (1 H, br s, N(3)-H), 7.06 and 6.78 (1 H and 2 H, dd and m, $J$ = 2.4 and 7.0 Hz for dd, aromatic protons, no assignments made), 6.14 and 5.10 (4 H, 2 br s, C(2)- and C(8)-amino protons, no assignments made). Addition of $D_2O$ resulted in collapse of the N(3)-, C(2)-, and C(8)-protons.

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**Registry No.** 1, 130148-30-0; 2, 130148-31-1; 2 Na salt, 130148-56-0; 2 benzoylated, 130148-57-1; 3, 105664-88-8; 6, 130148-32-2; 7, 130148-33-3; 8, 130148-34-4; 9, 130148-35-5; 10, 130148-36-6; 11, 130148-37-7; 12, 130148-38-8; 13, 130148-39-9; 14, 130148-40-2; 15, 130148-41-3; 16, 130148-42-4; 19H, 130148-59-3; 20, 53638-54-3; 21, 130148-46-8; 22, 130148-47-9; 23, 130148-48-0; 24, 130148-49-1; 24 2-amino derivative, 130148-52-6; 25, 130148-50-4; 26, 130148-58-2; PNPase, 9030-21-1; xanthine oxidase, 9002-17-9; imidazo[4,f-g]quinazolin-8(3*H*,7*H*)-one, 53449-18-6; β-D-ribofuranose-1,2,3,5-tetra-*o*-acetate, 13035-61-5; 5-nitrobenzimidazole-6-carboxylic acid, 130148-51-5; 6-(*N*-methylcarbamyl)-5-nitrobenzimidazole, 130148-43-5; 7-methylimidazo[4,5-g]quinazolin-8(3*H*,7*H*)-one, 130148-44-6; 7-methyl-4-nitroimidazo[4,5-g]quinazolin-8(3*H*,7*H*)-one, 130148-45-7; 1-*o*-acetyl-2,3,5-tri-*o*-benzoyl-β-D-ribofuranose, 6974-32-9; 2-amino-5-bromobenzoic acid, 5794-88-7; 2-amino-6-bromoquinazolin-3-(4*H*)-one, 130148-53-7; 2-amino-6-bromo-8-nitroquinazolin-4-(3*H*)-one, 130148-54-8; 2,8-diaminoquinazolin-4(3*H*)-one dihydrochloride, 130148-55-9.