mmol) was added dropwise during 10 min and the solution was stirred at −78 °C for 20 min. The solution temperature was adjusted to 0 °C (ice/water bath) and gaseous formaldehyde (generated by heating paraformaldehyde to 150–160 °C in a stream of nitrogen and dried by passing over phosphorus pentoxide) was bubbled into the solution until a straw-colored solution was obtained. The solution was stirred overnight at room temperature and was then poured into saturated ammonium chloride solution (50 mL) and extracted with ether (5 × 10 mL). The ether extracts were washed with water (10 mL), saturated $NaHCO_3$ (2 × 10 mL), and brine (10 mL) and dried ($MgSO_4$). Evaporation of solvents gave 2.39 g of residue, which was chromatographed on silica gel to give, after evaporation of solvent and kugelrohr distillation, 0.339 g (34%) of epi-*cis*-β-santalol (**9**), bp 110–120 °C (0.3 mm), 91% pure. Further purification gave material which was 96% pure: NMR ($CDCl_3$) δ 1.01 (3 H, s, $>CCH_3$), 1.79 (3 H, br s, $=C(CH_2OH)CH_3$), 1.0–2.3 (12 H, m), 2.60–2.75 (1 H, m, $CHC=CH_2$), 4.14 (2 H, s, CH_2OH), 4.46 and 4.73 (2 H, 2 s, $>C=CH_2$), 5.15–5.35 (1 H, m, $CH=C<$); IR (film) 3330, 2940, 1660 cm^{-1}; mass spectrum, *m/e* 220, 202, 187, 159. Anal. Calcd for $C_{15}H_{24}O$: C, 81.76; H, 10.98. Found: C, 81.48; H, 10.91.

(*E*)-Ethyl 2-Methyl-5-(2-*exo*-methyl-3-methylenebicyclo[2.2.1]hept-2-yl)-2-pentenoate (30). Sodium hydride (0.212 g of a 50% oil dispersion, 4.42 mmol) was washed with dimethoxyethane (3 × 5 mL). To a suspension of the sodium hydride in dimethoxyethane (25 mL) was added triethyl phosphonopropanoate (1.052 g, 4.42 mmol) in dimethoxyethane (5 mL). When hydrogen evolution had ceased, the solution was cooled (0 °C) and aldehyde **23** (0.786 g, 4.42 mmol, 86% pure) in dimethoxyethane (5 mL) added dropwise during 5 min. The mixture was stirred at 25 °C for 1 h, then heated at 60–70 °C for 30 min, cooled, poured into water (30 mL), and extracted with ether (4 × 10 mL). The ether extracts were washed with water (3 × 10 mL) and then brine (10 mL) and dried ($MgSO_4$). The solvents were evaporated and the residue was chromatographed on silica gel to give, after evaporation of solvent and kugelrohr distillation, 0.773 g (67%) of esters, bp 110–115 °C (0.3 mm). GLC analysis indicated 81% *E* and 9% *Z* ester **30**: NMR ($CDCl_3$) δ 1.03 (3 H, s, $>CCH_3$), 1.15–1.38 (3 H, t, J = 7 Hz, CH_2CH_3), 1.85 (3 H, br s, $=C(CO_2R)CH_3$), 1.0–2.4 (11 H, m), 2.6–2.8 (1 H, m, $CHC=CH_2$), 4.00–4.37 (2 H, q, J = 7 Hz, CH_2CH_3), 4.48 and 4.73 (2 H, 2 s, $>C=CH_2$), 6.6–7.0 (1 H, m, $CH=C(CO_2R)$); IR (film) 2960, 1705, 1650, 1460 cm^{-1}; mass spectrum, *m/e* 262, 247, 234, 216, 189, 161; UV (95% EtOH) 217 nm (calcd 217) (ε 16 300). Anal. Calcd for $C_{17}H_{26}O_2$: C, 77.81; H, 9.98. Found: C, 77.58; H, 10.10.

(*E*)-2-Methyl-5-(2-*exo*-methyl-3-methylenebicyclo[2.2.1]hept-2-yl)-2-penten-1-ol (Epi-*trans*-β-santalol) (10). To a cold (0 °C) solution of aluminum chloride (0.089 g, 0.67 mmol) in ether (10 mL) was added portionwise lithium aluminum hydride (0.076 g, 2 mmol). The solution was stirred for 30 min at 0 °C and then esters **30** (0.262 g, 1 mmol; 82% *E*, 14% *Z*) in ether (3 mL) were added dropwise. The solution was stirred at 0 °C for 1.3 h and then cautiously poured into 2 N HCl (5 mL) and ice (5 mL). The aqueous layer was extracted with ether (4 × 10 mL). The combined organic extracts were washed successively with water (2 × 5 mL), saturated $NaHCO_3$ (3 × 5 mL), and brine (5 mL) and dried ($MgSO_4$). Evaporation of solvent and kugelrohr distillation gave 0.191 g (87%) of a colorless oil, bp 120–130 °C (0.3 mm). GLC analysis indicated 83% epi-*trans*-β-santalol (**10**) and 14% epi-*cis*-β-santalol (**9**). A sample for 80-MHz ^1H NMR analysis was obtained by preparative GLC: NMR ($CDCl_3$) δ 1.03 (3 H, s, $>CCH_3$), 1.69 (3 H, br s, $=C(CH_2OH)CH_3$), 1.0–2.3 (12 H, m), 2.60–2.75 (1 H, m, $CHC=CH_2$), 4.00 (2 H, s, CH_2OH), 4.47 and 4.72 (2 H, 2 s, $>C=CH_2$), 5.3–5.6 (1 H, m, $CH=C(CH_2OH)$); IR (film) 3300, 2960, 1660 cm^{-1}; mass spectrum, *m/e* 220, 205, 202, 187, 159. Anal. Calcd for $C_{15}H_{24}O$: C, 81.76; H, 10.98. Found: C, 81.61; H, 10.95.

2-Methyl-5-(2-*exo*-methyl-3-methylenebicyclo[2.2.1]hept-2-yl)pentan-1-ol (Dihydroepi-β-santalol) (11). Lithium shot was added to a mixture of esters **30** (0.262 g, 1 mmol), ethanol (5 mL), ether (5 mL), and ammonia (20 mL) until a persistent blue color was observed. Ammonium chloride (2 g) was added and ammonia allowed to evaporate. The residue was dissolved in ether (20 mL) and water (10 mL). The aqueous layer was extracted with ether (3 × 5 mL), and the ether extracts were washed successively with water (2 × 5 mL) and brine (5 mL) and dried ($MgSO_4$). The solvents were evaporated and the residue was chromatographed on silica gel to give after kugelrohr distillation 0.155 g (70%) of a colorless oil, bp 110–130 °C (0.3 mm). GLC analysis indicated a purity of 99%: NMR (CCl_4) δ 0.84–0.96 (3 H, d, J = 7 Hz, $>CHCH_3$), 0.99 (3 H, s, CH_3), 0.8–2.2 (14 H, m), 2.63 (1 H, s, OH), 2.5–2.75 (1 H, m, $CHC=CH_2$), 3.33–3.42 (2 H, d, J = 6 Hz, CH_2OH), 4.42 and 4.66 (2 H, 2 s, $>C=CH_2$); IR (film) 3350, 2960, 1660 cm^{-1}; mass spectrum, *m/e* 222, 207, 204, 161, 149. Anal. Calcd for $C_{15}H_{26}O$: C, 81.02; H, 11.79. Found: C, 80.62; H, 11.57.

Acknowledgment. Valuable discussions with Professor Sir Derek Barton are gratefully acknowledged.

Registry No. 3, 512-61-8; **6**, 6090-26-2; **7**, 69685-57-0; **8**, 37876-51-0; **9**, 73855-14-8; **10**, 73890-74-1; **11**, 34289-89-9; **18**, 73838-28-5; **19**, 73855-15-9; **20**, 73838-29-6; **21**, 69667-88-5; **22**, 73838-30-9; **23**, 73838-31-0; **23** semicarbazone, 73838-32-1; **24**, 73855-16-0; **28**, 69652-67-1; **29**, 73890-05-8; (*E*)-**30**, 73838-33-2; (*Z*)-**30**, 73838-34-3; **31**, 69668-75-3.

Synthesis of *prox*-Benzoisoallopurinol

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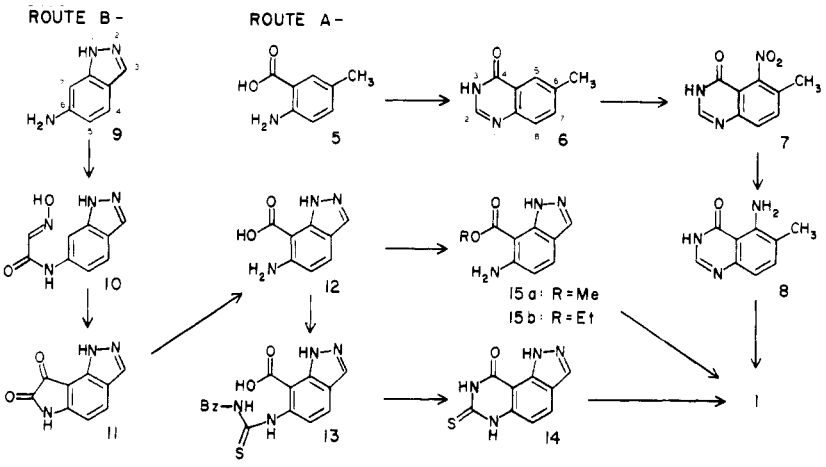
Pyrazolo[3,4-*f*]quinazolin-9-one (*prox*-benzoisoallopurinol, **1**), an extended analogue of 7-hydroxypyrazolo[4,3-*d*]pyrimidine (isoallopurinol, **2**) and a potential dimensional probe for substrates of xanthine oxidase, has been synthesized by two independent routes. The title compound, prepared by elaboration of either a suitably substituted indazole or a quinazolinone, was found to be an active substrate for and an alternative-substrate inhibitor of xanthine oxidase. The product of enzymatic oxidation of *prox*-benzoisoallopurinol has been identified as the corresponding *prox*-benzoisoalloxanthine.

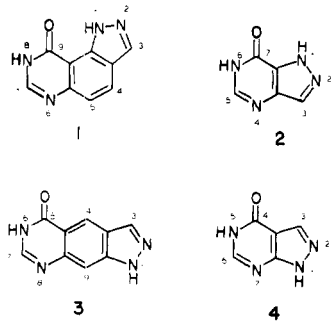
Recent work in this laboratory has centered on the synthesis of "stretched-out" versions of biologically active purine compounds.[1] Encouraged by the fluorescence and biochemical properties of extended analogues in this se-

0022-3263/80/1945-3072$01.00/0

Scheme I



ries,[2] we have initiated an investigation of similarly extended pyrazolopyrimidines, and we report here the synthesis of *prox*-benzoisoallopurinol (1), an extended ana-



logue of isoallopurinol (2) and the second in a series of benzoallopurinols. The synthesis of the first such compound, pyrazolo[4,3-*g*]quinazolin-5-one (*lin*-benzoallopurinol, 3), a 2.4-Å-wider version of allopurinol (4, the drug of choice for the treatment of hyperurecemia[3] and a potent inhibitor of xanthine oxidase), has recently been reported.[4] A commitment to the exploration of the dimensional requirements of enzyme active sites together with an interest in substrates and inhibitors of xanthine oxidase encouraged us to synthesize and test *prox*-benzoisoallopurinol.

Since *prox*-benzoisoallopurinol (1) can be perceived as either a fused quinazoline (Scheme I, route A) or a fused indazole (Scheme I, route B), two independent routes to its synthesis were envisioned. Because route A proceeds via the known quinazolinone 6[5] and route B via the known indazole 9,[6] the convergence of these independent synthetic routes provides unequivocal structure proof.

Synthesis. Route A. 5-Methylanthranilic acid (5)[6] was fused with formamide (the Niementowski procedure)[7] to give the known 6-methylquinazolin-4-one (6).[5] Nitration with potassium nitrate in sulfuric acid, a procedure which allows stoichiometric addition of the nitrating agent, gave the isomer 6-methyl-5-nitroquinazolin-4-one (7) as the sole mononitro product. This was readily reduced to the fused *o*-toluidine-type compound 8 with hydrazine over Raney nickel.

The treatment of compound 8 with sodium nitrite in acetic acid gave *prox*-benzoisoallopurinol (1) accompanied by many highly colored side products. The promoting effect of the pyrimidone ring of 8 toward indazole formation, reported to be necessary in this type of ring closure,[8] was insufficient for the clean formation of 1.

Route B. The availability of a substituted indazole (9)[6] directed investigation of a second route to *prox*-benzoisoallopurinol (1). The many documented routes to quinazolinones[9] suggested a pyrazoloanthranilic acid (i.e., 12) as a logical synthetic intermediate. The isatin 11[10] was synthesized in two steps from 6-aminoindazole (9) by using the Sandmeyer procedure.[11] Of the two possible isatin isomers that might have resulted from the ring closure of 10, we know that only the "bent" one (11) was formed, on the basis of the NMR spectra of the reaction products, in which no proton resonances attributable to a "linear" compound were observed. This result is consistent with the experiments of Burch,[12] in which the Conrad–Limpach[13] procedure yielded a *prox*-pyrazoloquinoline, and of Primc,[14] in which only "bent" isomers of pyrazoloquinolines were formed in the Skraup synthesis from 5- and 6-aminoindazole. Alkaline oxidation of the isatin 11 gave the substituted anthranilic acid 12.

Conversion of 12 into 1 was complicated by the facile

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Table I. Kinetic Data[a] from Oxidation of Purines and Purine Analogues by Xanthine Oxidase

compd	K_m[b]	V_{max}[c]	$K_{i,slope}$[b]
hypoxanthine	1.75×10^{-6}	3.87×10^{-7}	*d*
1	1.45×10^{-5}	7.32×10^{-7}	0.95×10^{-5}
3[e]	1.6×10^{-6}	2.67×10^{-7}	1.77×10^{-6} [f]
allopurinol	*g*	*g*	5.4×10^{-10} [h]

[a] Based on Lineweaver-Burk plots (least squares linear fit to data). [b] In units of moles per liter. [c] In units of moles per minute per milligram of protein. [d] Not applicable. [e] Reference 4. [f] Previously unpublished result. [g] Not measurable under these conditions.[27] [h] This is the reported[28b] dissociation constant for the enzyme-inhibitor complex and is more meaningful here than the $K_{i,slope}$ for allopurinol, which is 7.0×10^{-7} M.[27]

decarboxylation of **12** on heating to yield 6-aminoindazole (**9**). Early attempts to form the quinazolinone **1** from the anthranilic acid **12**, i.e., by heating with formamide, failed. A gentle method for quinazolinone synthesis involving the use of benzoyl isothiocyanate[15,16] was applied to generate compound **1**. The heating of compound **12** in acetone with a benzene solution of benzoyl isothiocyanate gave the thioureido compound **13**. The mercaptoquinazolinone **14**, formed by treatment of **13** with either aqueous hydroxide or ammonia at reflux, was subsequently reduced to *prox*-benzoisoallopurinol (**1**) by heating it in ammonia[17] over Raney nickel,[18] with the conformation of nickel salts.

Alternatively, target compound **1** could be synthesized via the ester of **12** (**15**). Methyl 6-aminoindazole-7-carboxylate (**15a**) was synthesized by the treatment of **12** with an anhydrous solution of diazomethane (**Caution!**)[19] prepared without distillation[20] from *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.[6] The ethyl ester **15b** was prepared similarly from **12** and *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine.[6,21] For preparations of greater than 100 mg of the ester, the method of Arndt[22] was convenient for the generation of diazoethane. Although the ethyl ester **15b** resisted fusion with formamide to form **1** and conversion with ammonia to the corresponding amide, it could be formylated with acetic formic anhydride[23] followed by heating in liquid ammonia at 120 °C to give crystalline **1** in analytically pure form. The preferred route for the preparation of **1** from **9** is thus **9** → **10** → **11** → **12** → **15b** → **1**. *prox*-Benzoisoallopurinol (**1**) was found to be nonfluorescent.

Behavior with Xanthine Oxidase. We have investigated the behavior of *prox*-benzoisoallopurinol (**1**) both as a substrate and as an inhibitor with xanthine oxidase.[24]

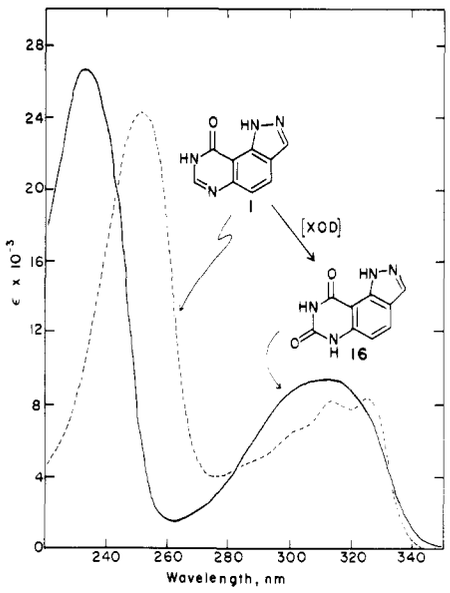
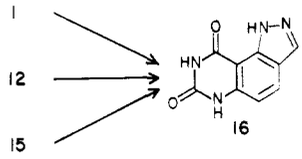


Figure 1. Ultraviolet absorption spectra of *prox*-benzoisoallopurinol (**1**) (---) and its xanthine oxidase product **16** (—) in phosphate buffer at pH 7.8.

Scheme II



Using conventional assay methods with oxygen as the final electron acceptor,[25] we obtained data as reported in Table I. Because *prox*-benzoisoallopurinol is a substrate for xanthine oxidase (Figure 1), it was hypothesized that its mode of inhibition might be that of an alternative substrate.[26] We observed the oxidation of hypoxanthine (as uric acid formation) in the presence and absence of 1.4×10^{-5} M *prox*-benzoisoallopurinol (**1**) over a range of hypoxanthine concentrations. A Lineweaver–Burk plot (least-squares linear fit to data) showed that the inhibition was competitive and that, consistent with an alternative substrate mode of inhibition,[26] $K_{i,slope} = K_m$ within experimental error. To test the possibility of inhibition by the oxidation product of **1**, analogous to the development of inhibition by the oxidation product of allopurinol,[27] we preincubated samples of xanthine oxidase alone in a buffer with compound **1** or with allopurinol (**4**) to determine whether inhibition developed. The *prox*-benzoisoallopurinol-treated enzyme oxidized hypoxanthine at the same rate as the untreated enzyme, whereas the allopurinol-treated enzyme lost nearly all its catalytic ability. These results parallel those obtained with *lin*-benzoallopurinol (**3**), which also inhibits xanthine oxidase competitively by an alternative substrate mode.[4]

Like allopurinol (**4**),[27] both benzoallopurinols **1** and **3** are excellent substrates for xanthine oxidase, with activities

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(19) **Caution**: Diazoalkanes are explosive and toxic. All manipulations should be carried out in an efficient fume hood. Gloves should be worn. See: Sax, N. I. "Dangerous Properties of Industrial Materials", 4th ed.; Van Nostrand-Reinhold: New York, 1975; p 610.

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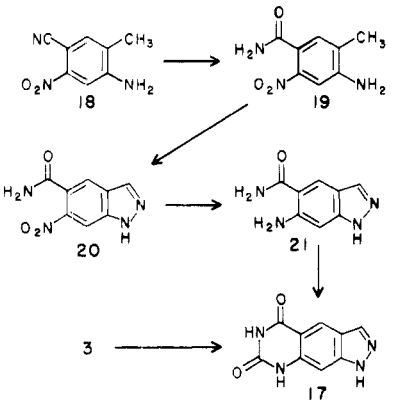
Scheme III



comparable to those of the natural substrate hypoxanthine. Unlike allopurinol, compounds **1** and **3** do not inhibit xanthine oxidase by a powerful secondary mechanism[28] and are inhibitors only in concentrations comparable to their K_m's. These results indicate that while both **1** and **3** are relatively unpromising xanthine oxidase inhibitors, both bent (**1**) and linear (**3**) extended analogues of isoallopurinol or allopurinol (**4**) can be accommodated satisfactorily at the active site of xanthine oxidase.

We have identified the products of oxidation of **1** and of **3** by xanthine oxidase. *prox*-Benzoisoalloxanthine (pyrazolo[3,4-*f*]quinazoline-7,9-dione, **16**) was synthesized chemically from compound **12** by treatment with benzoyl isocyanate[29] followed by ring closure in aqueous hydroxide or from intermediate **15** by treatment with ethyl chloroformate followed by ring closure with liquid ammonia at 120 °C (Scheme II). For enzymatic synthesis, compound **1** in dimethyl sulfoxide was diluted in aqueous buffer and oxidized with xanthine oxidase. The solid obtained after evaporation of the buffer and precipitation from the remaining dimethyl sulfoxide was identical in all respects with chemically prepared **16**, as judged by "mixed NMR" spectra and other properties.

In an analogous experiment, the enzymatic oxidation product of *lin*-benzoallopurinol (**3**)[4] has been identified by chemical synthesis (Scheme III). The substituted benzonitrile **18**, which we have described previously,[4] was hydrolyzed in sulfuric acid to the nitro amide **19**, from which the indazole **20** was formed by treatment with sodium nitrite in acetic acid. The reduction of intermediate **20** with hydrazine over Raney nickel afforded **21**, which was fused with urea to give *lin*-benzoalloxanthine (pyrazolo[4,3-*g*]quinazoline-5,7-dione, **17**), identical in all respects with the product obtained from the enzyme-catalyzed oxidation of **3**. Neither compound **16** nor compound **17** was oxidized further by xanthine oxidase.

Experimental Section

Unless otherwise indicated, all thin-layer chromatographic (TLC) separations were performed on Merck precoated silica gel f-254 plates with fluorescent backing and were developed with ethyl acetate. Melting points were determined on a Büchi melting point apparatus and are uncorrected. Reactions in ammonia were performed in a high-pressure bomb obtainable from the Parr Instrument Co. The NMR spectra were recorded on a Varian Associates EM-390 or HA-220 spectrometer using tetramethylsilane as an internal standard. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), integration, coupling constants, and assignment. Mass spectra were obtained by Mr. J. Carter Cook and his staff on a Varian MAT CH-5 low-resolution or a Varian MAT-731 high-resolution spectrometer coupled with a 620i computer and a STATOS recorder. Ultraviolet absorption spectra were obtained on a Beckman Acta MVI spectrometer, and the maxima are reported as follows: solvent, wavelength, absorption coefficient, and description (sh = shoulder). Microanalyses were performed by Mr. Josef Nemeth and his staff or by Midwest Microlab, Ltd. Raney nickel (No. 28) was used as purchased from the Grace Chemical Co. Buttermilk xanthine oxidase (xanthine oxygen oxidoreductase; EC No. 1.2.3.2) was purchased from Sigma Chemical Co.

Pyrazolo[3,4-*f*]quinazolin-9-one (*prox*-Benzoisoallopurinol, 1). From Ethyl 6-Aminoindazole-7-carboxylate (15b). An ice-cold, stirred solution of 400 mg (1.95 mmol) of ethyl 6-aminoindazole-7-carboxylate (**15b**) in 10 mL of 98% formic acid was treated with 5 mL of acetic formic anhydride[23] at 0 °C and allowed to reach room temperature. When TLC (developed in 50:50 ethyl acetate/hexane) showed that all of the starting material had been consumed, the solvents were evaporated in vacuo, and the residue was freed of residual solvents by the azeotropic evaporation of aliquots of dry toluene (3 × 10 mL) in vacuo. The solid was placed in a 110 mL stainless-steel reaction bomb, cooled in a dry ice/acetone bath, and covered with 20 mL of liquid ammonia. The bomb was sealed, heated to 120 °C for 12 h, and cooled slowly to room temperature before being reimmersed in a dry ice/acetone bath and unsealed. The ammonia was decanted, leaving 200 mg (51%) of tan, analytically pure needles of **1**: mp >300 °C; NMR ($(CD_3)_2SO$) δ 7.34 (d, 1, J = 9 Hz, $C_{ar}H$), 8.12 (d, 1, J = 9 Hz, $C_{ar}H$), 8.19 (s, 2, $C_{ar}H$); mass spectrum, m/e 186 (M^+); UV max (phosphate buffer, pH 7.8) 252 nm (ε 24600), 314 (8300), 326 (8400).

Anal. Calcd for $C_9H_6N_4O{\cdot}NH_3$: C, 53.19; H, 4.46; N, 34.47. Found: C, 52.99; H, 4.37; N, 34.67.

From 5-Amino-6-methylquinazolin-4-one (8). A solution of 140 mg (0.8 mmol) of 5-amino-6-methylquinazolin-4-one (**8**) in 15 mL of glacial acetic acid, treated at once with 55 mg (0.8 mmol) of sodium nitrite in 1 mL of water, was stirred at 20 °C for 3 days. The solvents were evaporated in vacuo, and the residual solid was triturated with water and filtered to give, after drying, a brown solid (50 mg, 34%; mp >300 °C) spectroscopically identical with that prepared by the first route. The absence of NH_3 in this case and the one below did not interfere in the UV or NMR spectra.

From 7-Mercaptopyrazolo[3,4-*f*]quinazolin-9-one (14). A hot solution of 7-mercaptopyrazolo[3,4-*f*]quinazolin-9-one (**14**, 100 mg, 0.45 mmol) in 20 mL of 5 M ammonium hydroxide was heated at reflux over Raney nickel for 1.5 h. The catalyst was removed by filtration, and the solvents were removed in vacuo to give 75 mg (90%) of a pale powder which contained some nickel salts: mp >300 °C, spectroscopically identical with that prepared by the other routes.

6-Methyl-5-nitroquinazolin-4-one (7). A solution of 1.0 g (6.2 mmol) of 6-methylquinazolin-4-one (**6**)[5] in 10 mL of concentrated sulfuric acid was added to a solution of 630 mg (6.25 mmol) of potassium nitrate in 10 mL of concentrated sulfuric acid. The combination was allowed to stand at room temperature for 24 h before being poured over 200 mL of crushed ice. The precipitate was collected and dried to give 830 mg (65%) of a pale yellow powder: mp 265 °C dec; NMR ($(CD_3)_2SO$) δ 2.30 (s, 3, CH_3), 7.75 (d, 1, J = 9 Hz, $C_{ar}H$), 7.88 (d, 1, J = 9 Hz, $C_{ar}H$), 8.20 (s, 1, 2-H); mass spectrum, m/e 205 (M^+); exact mass calcd for $C_9H_7N_3O_3$ 205.0487, found 205.0485.

5-Amino-6-methylquinazolin-4-one (8). A solution of 3.0 g (14.6 mmol) of 6-methyl-5-nitroquinazolin-4-one (**7**) in 200 mL of absolute ethanol (heating may be required to effect solution) was treated at 0 °C with 200 mg of 10% palladium on charcoal catalyst. Stirring was initiated, and 2.5 mL of 98% hydrazine hydrate was added dropwise while the suspension was allowed to warm to room temperature, where it remained for 1 h before being heated to reflux for an additional 2 h. The suspension was filtered hot to remove the catalyst, and the filtrate was evaporated in vacuo to give 2.2 g (86%) of a tan solid: mp 212 °C dec; NMR ($(CD_3)_2SO$) δ 2.10 (s, 3, CH_3), 6.63 (d, 1, J = 9 Hz, 8-H), 6.92 (s, NH), 7.29 (d, 1, J = 9 Hz, 7-H), 7.80 (s, 1, 2-H); mass spectrum,

(28) (a) Spector, T.; Johns, D. G. *J. Biol. Chem.* **1970**, *245*, 5079. (b) Massey, V.; Komai, H.; Palmer, G.; Elion, G. B. *J. Biol. Chem.* **1970**, *245*, 2837.

(29) Speziale, A. J.; Smith, L. R.; Fedder, J. E. *J. Org. Chem.* **1965**, *30*, 4306.

m/e 175 (M^+); exact mass calcd for $C_9H_9N_3O$ 175.0744, found 175.0743.

6-(Isonitrosoacetamido)indazole (10). Into a 1-L flask equipped with a mechanical stirrer and condenser were placed, in order, 9.0 g (54 mmol) of chloral hydrate,[30] 117 mL of water, 130 g of sodium sulfate, a solution of 6.65 g (50 mmol) of 6-aminoindazole (**9**)[6] in 185 mL of water containing 4.3 mL of concentrated hydrochloric acid, and a solution of 18 g (130 mmol) of hydroxylamine hydrochloride in 32 mL of water. The mixture was heated slowly to gentle reflux over 4 h, maintained at reflux for 30 min, and immersed in an ice bath. The resulting tan precipitate was filtered, triturated with 250 mL of hot water, refiltered, and dried to yield 10.2 g (100%) of a crude beige solid that could be used in the next step without further purification: mp 200 °C dec; NMR ($(CD_3)_2SO$) δ 7.23 (d, 1, J = 9 Hz, $C_{ar}H$), 7.62 (d, 1, J = 9 Hz, $C_{ar}H$), 7.67 (s, 1), 7.97 (s, 1), 8.17 (s, 1), 10.27 (s, 1); mass spectrum, m/e 204 (M^+); exact mass calcd for $C_9H_8N_4O_2$ 204.0647, found 204.0642.

Pyrrolo[2,3-*g*]indazole-7,8-dione (11). To 10 mL of concentrated sulfuric acid was added 2.5 g (12.3 mmol) of moisture-free 6-(isonitrosoacetamido)indazole (**10**) with vigorous stirring to prevent local heating. The resulting solution was warmed at 80 °C in an oil bath for 30 min and then poured over 250 mL of crushed ice. The solid which separated was isolated by filtration and taken up in 50 mL of 2 M sodium hydroxide, and this mixture was filtered to remove impurities and neutralized carefully with glacial acetate acid. The resulting suspension was filtered to remove further impurities, and the filtrate was acidified carefully with concentrated hydrochloric acid. The red solid which separated was filtered and dried to give 2.02 g (88%) of crude **11**: mp 300 °C; NMR ($(CD_3)_2SO$) δ 6.73 (d, 1, J = 9 Hz, $C_{ar}H$), 8.08 (s, 1, 3-H), 8.08 (d, 1, J = 9 Hz, $C_{ar}H$), 11.7 (s); mass spectrum, m/e 187 (M^+).

Anal. Calcd for $C_9H_5N_3O_2$: C, 57.76; H, 2.69; N, 22.45. Found: C, 57.48; H, 2.60; N, 22.18.

6-Aminoindazole-7-carboxylic Acid (12). At no time during this procedure[11c] should the temperature be allowed to exceed 60 °C. A 250-mL flask fitted with a mechanical stirrer and a thermometer was charged with a solution of 5.3 g (216 mmol) of pyrrolo[2,3-*g*]indazole-7,8-dione (**11**) in 100 mL of 1.5 M aqueous sodium hydroxide and warmed to 50 °C. Hydrogen peroxide (30%, 7.0 mL) was added dropwise to the reaction, which must be kept between 50 and 60 °C. The reaction was initially exothermic enough to maintain the desired temperature without external heating. When the addition was complete, the mixture was stirred overnight at room temperature. The mixture was neutralized slowly and carefully with concentrated hydrochloric acid, and this mixture was stirred at room temperature for 30 min with activated charcoal, filtered through Celite, concentrated in vacuo to 30 mL, and brought to pH 4 by further addition of concentrated hydrochloric acid. The suspension was cooled to 0 °C and filtered, and the residue was dried at room temperature, affording 3.62 g (72%) of a tan powder: mp 110 °C dec; NMR ($(CD_3)_2SO$) δ 6.60 (d, 1, J = 9 Hz, $C_{ar}H$), 7.57 (d, 1, J = 9 Hz, $C_{ar}H$), 7.83 (s, 1, 3-H); mass spectrum, m/e 177 (M^+); exact mass calcd for $C_8H_7N_3O_2$ 177.0538, found 177.0540.

6-(β-Benzoylthioureido)indazole-7-carboxylic Acid (13). A suspension of 177 mg (1.0 mmol) of 6-aminoindazole-7-carboxylic acid (**12**) in 15 mL of acetone was heated at reflux with 0.5 mL of a 2 M solution of benzoyl isothiocyanate in benzene[15b] for 12 h. The beige suspension thus formed was filtered, and the residue was dried, giving 150 mg (44%) of a tan solid. An analytical sample could be obtained by recrystallization from ethanol/dimethylformamide: mp 210 °C dec, NMR ($(CD_3)_2SO$) δ 7.54 (m, 3, $C_{ar}H$), 7.83 (s, 1), 7.98 (m, 3, $C_{ar}H$), 8.14 (s, 1), 11.48 (s, 1), 13.18 (s, 1); mass spectrum, m/e (field desorption) 340 (M^+).

Anal. Calcd for $C_{16}H_{12}N_4O_3S$: C, 56.46; H, 3.55; N, 16.46. Found: C, 56.32; H, 3.55; N, 16.44.

7-Mercaptopyrazolo[3,4-*f*]quinazolin-9-one (14). A suspension of 110 mg (0.32 mmol) of 6-(β-benzoylthioureido)-indazole-7-carboxylic acid (**13**) in 2 mL of 10% aqueous sodium hydroxide was heated at reflux for 4 h. Following cooling and careful neutralization with concentrated hydrochloric acid, the suspension was filtered, giving 60 mg (88%) of a tan solid, an analytical sample of which could be obtained by recrystallization from ethanol/dimethylformamide: mp >300 °C dec; NMR ($(CD_3)_2SO$) δ 7.12 (d, 1, J = 9 Hz, $C_{ar}H$), 8.09 (d, 1, J = 9 Hz, $C_{ar}H$), 8.14 (s, 1, 3-H); mass spectrum, m/e 218 (M^+).

Anal. Calcd for $C_9H_6N_4OS$: C, 49.53; H, 2.86. Found: C, 49.21; H, 2.77.

An identical sample was obtained by substituting concentrated ammonia for aqueous hydroxide.

Methyl 6-Aminoindazole-7-carboxylate (15a). Caution: *This procedure should be carried out in an efficient fume hood.*[19] In the procedure described by Fales et al.,[20] 400 mg (2.72 mmol) of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine[6] was decomposed 100 mg at a time, and the combined ethereal solutions were added to an ice-cold suspension of 80 mg (0.45 mmol) of 6-aminoindazole-7-carboxylic acid (**12**) in 30 mL of acetone. The suspension was stirred at 0 °C for 2 h and allowed to come to room temperature overnight. When TLC showed that all of the starting material had reacted, the solvents were evaporated with a stream of N_2 to yield a red powder: mp 175 °C; NMR ($CDCl_3$) δ 4.00 (s, 3, CH_3), 6.47 (d, 1, J = 9 Hz, 5-H), 7.58 (d, 1, J = 9 Hz, 4-H), 7.86 (s, 1, 3-H); mass spectrum, m/e 191 (M^+); exact mass calcd for $C_9H_9N_3O_2$ 191.0693; found 191.0694.

Ethyl 6-Aminoindazole-5-carboxylate (15b). Caution: *This procedure should be carried out in an efficient fume hood.*[19] A benzene/toluene solution of diazoethane procedure by the method of Arndt[22] from 300 mg (1.86 mmol) of *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine,[6] was added to a stirred suspension of 150 mg (0.85 mmol) of 6-aminoindazole-7-carboxylic acid (**12**) in 15 mL of acetone at 0 °C. The suspension was allowed to come to room temperature and was then filtered. The filtrate was evaporated with a stream of N_2 to give 140 mg (80%) of an orange solid which could be purified by sublimation: mp 180–181 °C; NMR ($(CD_3)_2SO$) δ 1.37 (t, 3, J = 7 Hz, CH_3), 4.42 (q, 2, J = 7 Hz, CH_2), 6.58 (d, 1, J = 9 Hz, 5-H), 7.54 (d, 1, J = 9 Hz, 4-H), 7.85 (s, 1, 3-H); mass spectrum, m/e 205 (M^+).

Anal. Calcd for $C_{10}H_{11}N_3O_2$: C, 58.53; H, 5.40; N, 20.48. Found: C, 58.72; H, 5.45; N, 20.67.

Pyrazolo[3,4-*f*]quinazoline-7,9-dione (*prox*-Benzoisoalloxanthine, 16). From 6-Aminoindazole-7-carboxylic Acid (12). A suspension of 177 mg (1.0 mmol) of 6-aminoindazole-7-carboxylic acid (**12**) in 15 mL of dry acetone was heated at reflux for 15 min with 1 g of 4-Å molecular sieves before being treated with 1 mL of a 1.0 M solution of benzoyl isocyanate[29] in benzene. The reaction was stirred at room temperature for 1 h, and the precipitate which had formed was isolated by filtration and then suspended and heated at reflux in 10 mL of 10% sodium hydroxide for 6 h. The suspension was filtered while hot, and the filtrate was neutralized with concentrated hydrochloric acid and filtered to give 113 mg (56%) of a beige powder: mp >250 °C; NMR ($(CD_3)_2SO$) δ 6.98 (d, 1, J = 9 Hz, $C_{ar}H$), 7.97 (d, 1, J = 9 Hz, $C_{ar}H$), 8.06 (s, 1, 3-H); mass spectrum, m/e 202 (M^+); exact mass calcd for $C_9H_6N_4O_2$ 202.0491, found 202.0491.

From Ethyl 6-Aminoindazole-7-carboxylate (15b). A suspension of 20 mg (0.1 mmol) of ethyl 6-aminoindazole-7-carboxylate (**15b**) in 10 mL of ethyl chloroformate was heated at reflux for 2 h. The solvent was removed in vacuo, and the residual solid was placed in a 110-mL stainless-steel bomb, cooled in a dry ice/acetone bath, and covered with 20 mL of ammonia. The bomb was sealed, heated to 120 °C for 12 h, recooled in a dry ice/acetone bath, and opened. Evaporation of the ammonia (effected by allowing the bomb to warm up slowly) gave 15 mg (80%) of a pale solid, identical with compound **16** prepared by the first route.

From Pyrazolo[3,4-*f*]quinazolin-9-one (1). A solution of 10 mg (0.53 mmol) of pyrazolo[3,4-*f*]quinazolin-9-one (**1**) in 0.5 mL of dimethyl sulfoxide was added dropwise to 200 mL of boiling water. The resulting solution was cooled to room temperature and mixed with 25 mL of 0.5 M TEAB[31] buffer (pH 7.7). But-

(30) In the first of these steps, chloral hydrate is required. This material is a restricted substance, and clearance from the U.S. Drug Enforcement Agency is required for its purchase.

(31) TEAB is triethylammonium bicarbonate. A 6-L sample was prepared as follows. At 0 °C, combine 425 mL of distilled triethylamine with 3 L of water in a 6-L flask, bubble carbon dioxide through this overnight, adjust the volume to 6 L with water, store the mixture at 0 °C, and check the pH. If the pH is >8, add more carbon dioxide.

termilk xanthine oxidase (250 μL of a 20 mg/mL suspension in 2.3 M ammonium sulfate) was pelletted in a centrifuge, dissolved in 1 mL of 0.5 M TEAB buffer, and added to the substrate. The course of the oxidation was monitored by UV spectrometry with 2-mm-path cells over the range 350–220 nm. When the reaction was complete, the solvents were concentrated in vacuo to 0.5 mL and treated with 10 mL of cold water, giving a precipitate which was isolated by centrifugation. The pellet was resuspended in acetone and repelletted, and the solid was dried in air to give 11 mg (98%) of a white solid, which was identical in all respects, including "mixed NMR" comparison, with that prepared by the chemical routes.

Pyrazolo[4,3-*g*]quinazoline-5,7-dione (17). From 6-Aminoindazole-5-carboxamide (21). A mixture of 200 mg (1.17 mmol) of 6-aminoindazole-5-carboxamide (**21**, preparation described below) and 400 mg (6.67 mmol) of urea was heated in an oil bath at 14 °C for 45 min. The clear solution partially solidified, and heating was continued at 180 °C for an additional 1.5 h, until the melt had become too solid to stir and the evolution of ammonia had ceased. The crude solid was triturated twice with 20 mL of water to give 85 mg (36%) of a tan solid: mp >300 °C; NMR ($(CD_3)_2SO$) δ 7.17 (s, 1, $C_{ar}H$), 8.19 (s, 1, $C_{ar}H$), 8.43 (s, 1, $C_{ar}H$); mass spectrum, *m/e* 202 (M^+); exact mass calcd for $C_9H_6N_4O_2$ 202.0490, found 202.0488.

From *lin*-Benzoallopurinol (3). A solution of 10 mg (0.053 mmol) of pyrazolo[4,3-*g*]quinazolin-5-one (*lin*-benzoallopurinol, **3**) in 0.5 mL of dimethyl sulfoxide was added dropwise to 250 mL of boiling water. The solution was cooled to 0 °C and mixed with 250 mL of 0.5 M TEAB[31] buffer (pH 7.7). Xanthine oxidase (500 μL of a 20 mg/mL suspension in 2.3 M ammonium sulfate) was pelletted in a centrifuge and dissolved in 1 mL of 0.5 M TEAB buffer after the supernatant was removed. The enzyme solution was added to the solution of **3** in TEAB, and the reaction mixture was allowed to come to room temperature. The reaction was monitored by UV spectrometry using 2-mm-path cells over the range 300–240 nm. When the reaction was complete (ca. 2 h), the solvents were concentrated in vacuo to 0.5 mL. Addition of 10 mL of cold water produced a precipitate which was isolated by centrifugation, resuspended in acetone, and repelletted in a centrifuge. The white solid thus obtained was dried in air to give 10 mg (92%) of compound **17** which was identical by "mixed NMR" and UV spectra with that prepared by the route described above.

4-Amino-5-methyl-2-nitrobenzamide (19). To 5 mL of 10% sulfuric acid was added 100 mg (0.56 mmol) of 4-amino-6-methyl-2-nitrobenzonitrile (**18**),[4] and the resulting suspension was placed in a 100 °C oil bath. Nitrogen was gently blown over the top of the suspension until the volume of the reaction mixture remained constant, at which time the nitrogen stream was removed, and the reaction was kept in the bath for an additional hour. The reaction mixture was poured over 80 g of crushed ice and neutralized carefully with concentrated ammonia. The yellow solid thus appearing was collected and dried to yield 100 mg (92%) of yellow crystals: mp 268 °C; NMR ($(CD_3)_2SO$) δ 2.10 (s, 1, CH_3), 5.75 (s, NH), 6.98 (s, 1, $C_{ar}H$), 7.24 (s, 1, $C_{ar}H$); mass spectrum, *m/e* 195 (M^+).

Anal. Calcd for $C_8H_9N_3O_3$: C, 49.23; H, 4.65; N, 21.53. Found: C, 49.39; H, 4.86; N, 21.30.

6-Nitroindazole-5-carboxamide (20). A suspension of 2.2 g (11.28 mmol) of very finely ground 4-amino-5-methyl-2-nitrobenzamide (**19**) in 220 mL of glacial acetic acid was treated all at once with a solution of 778 mg (11.3 mmol) of sodium nitrite in 2 mL of water, and the solution was stirred vigorously for 15 min before being allowed to stand at room temperature for 24 h. The solvents were evaporated in vacuo, and the residue was dried and used in subsequent steps without further purification: dec 226 °C; NMR ($(CD_3)_2SO$) δ 8.17 (s, 1, $C_{ar}H$), 8.27 (s, 1, $C_{ar}H$), 8.42 (s, 1, $C_{ar}H$); mass spectrum, *m/e* 206 (M^+).

6-Aminoindazole-5-carboxamide (21). A solution of 2.0 g (10 mmol) of 6-nitroindazole-5-carboxamide (**20**) in 600 mL of methanol was treated with 3 mL of 98% hydrazine hydrate. Raney nickel was added, and the suspension was stirred vigorously for 6 h and filtered through Celite to remove the catalyst. The filtrate was concentrated to 300 mL, treated with charcoal, and stripped of the solvents in vacuo to give 1.6 g (91%) of a tan solid which could be recrystallized from methanol: mp 268 °C (lit.[4] mp 265 °C); NMR ($(CD_3)_2SO$) δ 6.32 (s, NH), 6.57 (s, 1, 7-H), 7.81 (s, 1, $C_{ar}H$), 7.95 (s, 1, $C_{ar}H$); mass spectrum, *m/e* 176 (M^+).

Oxidation with Xanthine Oxidase[24] and Oxygen. The procedures used were modifications of the method of xanthine oxidase assay described by Boehringer Mannheim.[25a] Final assay mixtures had a total volume of 3.05 mL in a cuvette with a 1.0-cm light path. The assay mixtures contained oxygen as the final electron acceptor,[32] sodium EDTA at 0.10 mM, potassium phosphate buffer, pH 7.8 at 0.1 M, and the substrate to be oxidized at the specified concentrations. Each assay was initiated by addition of 50 μL of an enzyme solution which was a dilution of Sigma buttermilk xanthine oxidase (20 mg/mL suspension in 2.3 M ammonium sulfate) in 2 M ammonium sulfate in a ratio of 1:20 when compound **1** was the substrate and of 1:200 when hypoxanthine was the substrate. The oxidation of compound **1** was monitored at 254 nm, and the oxidation of hypoxanthine was monitored at 283 nm (the isosbestic point for the oxidation of **1**) as the formation of uric acid. Assays generally took 10–15 min for complete oxidation.

In a study of the possible inhibitory effect of *prox*-benzoisoallopurinol on uric acid formation from hypoxanthine, duplicate samples were run at each concentration, one with an inhibitor concentration of 1.4×10^{-5} M **1** and one without inhibitor. In a preincubation study, two 50-μL aliquots of 1:20 Sigma xanthine oxidase in 2 M ammonium sulfate were incubated with 450 μL of phosphate buffer or 450 μL of 2.9×10^{-5} M **1** in phosphate buffer, respectively, at 23 °C for 4 h. For each of the two sets of assays, 50 μL of this incubation mixture was used to catalyze the oxidation of 7.34×10^{-6} M hypoxanthine in phosphate buffer under the assay conditions described above, and the formation of uric acid was monitored as a function of time.

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Registry No. **1**, 73907-90-1; **3**, 71785-46-1; **6**, 19181-53-4; **7**, 73907-91-2; **8**, 73907-92-3; **9**, 6967-12-0; **10**, 73907-93-4; **11**, 73907-94-5; **12**, 73907-95-6; **13**, 73907-96-7; **14**, 73907-97-8; **15a**, 73907-98-9; **15b**, 73907-99-0; **16**, 73908-00-6; **17**, 73908-01-7; **18**, 71785-50-7; **19**, 73908-02-8; **20**, 73908-03-9; **21**, 71785-52-9; hydroxylamine hydrochloride, 5470-11-1; benzoyl isothiocyanate, 532-55-8.

(32) We found that the assay results were the same whether oxygen was bubbled into the assay mix before addition of enzyme or not.