Defined Dimensional Alterations in Enzyme Substrates. Synthesis and Enzymatic Evaluation of Some *lin*-Naphthopurines

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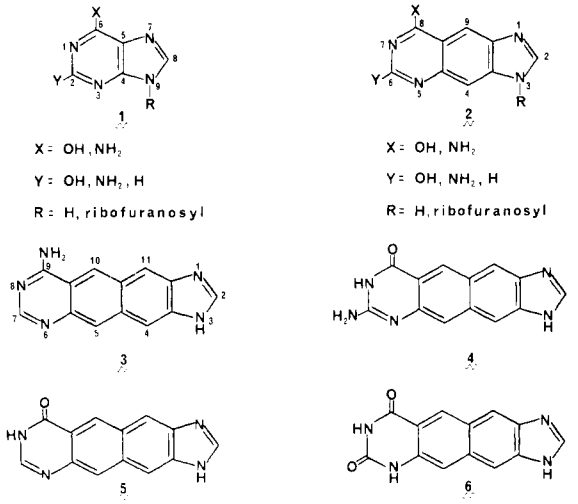
Abstract: The development of methodology for the regioselective syntheses of tetra-β-substituted naphthalenes via a combination of bicyclo[4.2.0]octa-1,3,5-triene and aryl trimethylsilyl chemistry led to the synthesis of benzimidazo[5,6-*g*]-6*H*,8*H*-quinazoline-7,9-dione (**6**) and benzimidazo[5,6-*g*]-8*H*-quinazolin-9-one (**5**), 4.8-Å laterally extended dimensional derivatives of xanthine and hypoxanthine. These compounds, *lin*-naphthoxanthine and *lin*-naphthohypoxanthine, exhibited intense fluorescence. *lin*-Naphthoxanthine was not oxidized to *lin*-naphthouric acid by xanthine oxidase but functioned as a noncompetitive inhibitor. However, *lin*-naphthohypoxanthine was readily converted to *lin*-naphthoxanthine by xanthine oxidase. In this reaction, *lin*-naphthohypoxanthine functioned as a competitive inhibitor of xanthine oxidase. The enzymatic results for the naphtho analogues when compared with the benzo analogues demonstrate, in part, a useful application of defined dimensional probes for determining the limiting spatial restrictions of the binding region for xanthine oxidase.

The challenge of setting the spatial restrictions of enzyme binding regions for purine-containing substrates or cofactors has led us to the development of defined dimensional probes.[1] These dimensional probes are purine ring analogues with lateral extensions of known magnitude, but with the peripheral rings intact. Parallel in vitro testing of naturally occurring purines **1** and *lin*-benzopurine derivatives **2** has produced an initial understanding



of the effects of altering the proximity of the terminal heterocyclic moieties on enzyme activity.[2] In an effort to define accurately the limiting restrictions of enzyme binding pockets, we have synthesized some benzimidazo[5,6-*g*]quinazolines (**3**–**6**) (tautomeric possibilities not restricted to those shown). These benzimidazoquinazolines, or *lin*-naphthopurine derivatives, have increased the interstitial distance of the *lin*-benzopurines by 2.4 Å (Figure 1), the same distance that these were extended over the purines.

Retrosynthetic analysis of the basic ring system offered three possible forward routes to it. (a) Removal of the terminal heterocyclic rings would require a tetra-β-substituted naphthalene intermediate. Naphthalene-based starting materials were essentially inappropriate due to the intrinsically unfavorable substitution pattern of naphthalene, that is, the α positions are the predominate sites for subtitution. Although some tetra-β-substituted naphthalenes have been described,[3,4,12] none was a suitable

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precursor for compounds **3–6**. Moreover, general methods have not been developed for the synthesis of systems of this type. (b) Several benzenoid systems have been employed to construct the naphthalene ring.[4d,h,dd,hh,ii,5] These systems would allow for the synthesis of tetra-β-substituted naphthalenes, but either the intermediates required or the projected reaction conditions precluded the formation of the imidazole ring. (c) An acyclic route would entail sequential ring formations and permit regioselective control of the construction of the desired naphthalene intermediates.

The goal of the synthetic methodology was twofold. First, the route should generate a general scheme for the regiospecific synthesis of naphthalenes. Second, the synthesis should provide an intermediate or intermediates amenable to conversion to all four of the nucleic acid base analogues (**3–6**).

Results and Discussion

The acyclic route that offered the greatest potential followed Vollhardt's application of the trimerization of acetylenes with cobalt catalysis.[6,7f] The product of interest from the trimerization was 3,4-bis(trimethylsilyl)bicyclo[4.2.0]octa-1,3,5-triene (**7**). Many syntheses of natural products[7c,e,f,i–l,n,o,8] and other polycyclic ring systems[7a,b,d,f–i,k,m–o,9] have employed the bicyclo[4.2.0]octa-1,3,5-triene or benzocyclobutene ring system as a precursor for an *o*-xylylene generated in situ. The stability of the four-membered ring under a variety of reaction conditions allowed greater latitude in the reaction sequence than did the use of other *o*-xylylene precursors.[7a,10]

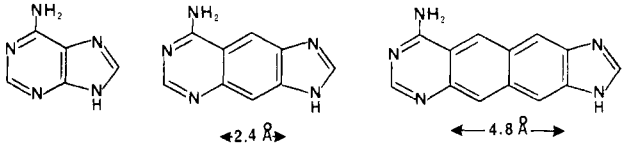

Figure 1.

The synthetic potential for electrophilic desilylation has been well established;[11] thus, the trimethylsilyl groups would allow for selective substitution (electrophilic desilylation) of two of the eventual four β positions of the naphthalene ring without competitive α substitution (electrophilic displacement of hydrogen). The reactivity of the aryl silyl groups provides the versatility of permitting the construction of the imidazole-ring precursor groups prior to the formation of the naphthalene nucleus. This sequence

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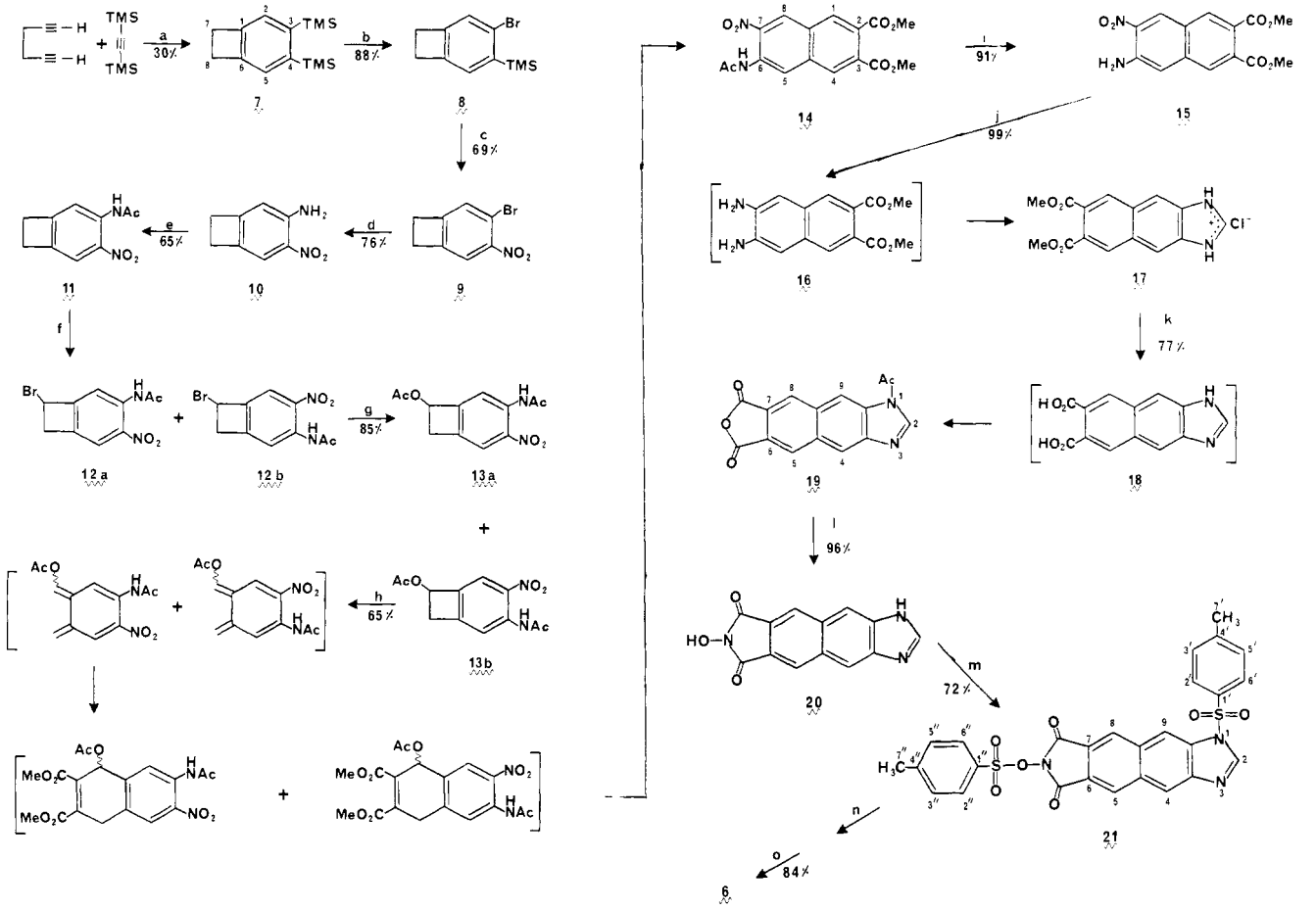
Scheme I[a]



[a] (a) $CpCo(CO)_2$; (b) Br_2, py, CCl_4; (c) HNO_3, Ac_2O, HOAc; (d) NH_3, *n*-BuOH, bomb; (e) AcCl; (f) NBS, CCl_4, $h\nu$, $(PhCO_2)_2$; (g) AgOAc, HOAc; (h) DMAD, st; (i) HCl, MeOH; (j) H_2, Pd/C, MeOH; $(MeO)_3CH$, HCO_2H; HCl, MeOH; (k) NaOH, MeOH; H_3O^+; Ac_2O; (l) $H_2NOH{\cdot}HCl$, py; (m) $(CH_3)_2SO$, DMAP, TsCl, py; (n) NH_3, EtOH; (o) NaOH.

of construction turned out to be crucial to success in the overall synthesis.

Bicyclooctatrienes could function as direct precursors for the naphthalene ring if the four-membered ring were appropriately substituted. For example, the Diels–Alder ($4_\pi + 2_\pi$) cycloaddition of 7-acetoxy-3,4-bis(trimethylsilyl)bicyclo[4.2.0]octa-1,3,5-triene, which is equivalent to an acetoxy-*o*-xylylene generated in situ, with bis(trimethylsilyl)acetylene gave 2,3,6,7-tetrakis(trimethylsilyl)naphthalene with loss of acetic acid.[12] Alternative chemical means were available to synthesize 7-acetoxy derivatives of bicyclooctatrienes. Application of this technology to our systems provided the key reaction sequence for the general synthesis of tetra-β-substituted naphthalenes (Scheme I).

Bromodesilylation of compound **7** with pyridinium perbromide gave 3-bromo-4-(trimethylsilyl)bicyclo[4.2.0]octa-1,3,5-triene (**8**) in good yield.[6] The second trimethylsilyl group was replaced by a nitro group under nitrodesilylation conditions chosen to minimize competing side reactions but retain the autocatalytic cycle.[13] The major product was 3-bromo-4-nitrobicyclo[4.2.0]octa-1,3,5-triene (**9**). The reaction mixture contained minor impurities caused by protodesilylation and fission of the four-membered ring by nitric acid.[14] Nitrodesilylation of bicyclooctatrienes proved superior to standard nitration methods for introduction of a nitro group into the aromatic ring.[15]

Nucleophilic displacement of the aryl bromide **9** with ammonia in a pressure vessel gave 3-amino-4-nitrobicyclo[4.2.0]octa-1,3,5-triene (**10**). Prolonged heating at 170–175 °C was necessary even though the bromine was ortho to a nitro group. the unreactive nature of the halide may result in part from the counteracting electron-donating capacity of the ethano bridge and in part from the elongated 3,4-bond, as in **7**.[16] The reaction temperature must not exceed 200 °C in order to prevent thermal ring opening of the four-membered ring. The thermal threshold for ($2_\pi + 2_\sigma$) cycloreversions of bicyclooctatrienes is dependent upon the substituent(s) on the four-membered ring. Electron-donating substituents facilitate thermal cycloreversions at lower temperature. In the case of the unsubstituted derivatives, thermal cycloreversions are first possible above 200 °C.[7i]

Acylation of amine **10** with acetyl chloride produced an N-protected intermediate, 3-acetamido-4-nitrobicyclo[4.2.0]octa-1,3,5-triene (**11**), which permitted subsequent attack exclusively on the four-membered ring. Radical bromination of **11** under drastic conditions failed to give complete reaction. The use of 1 equiv of *N*-bromosuccinimide gave mixtures of **11** and both brominated isomers, 3-acetamido-7-bromo-4- and 4-acetamido-7-bromo-3-nitrobicyclo[4.2.0]octa-1,3,5-triene (**12a + b**), in 50–60% yield. Additional equivalents of *N*-bromosuccinimide resulted in polybromination with recovery of **11** and a lowered yield of **12**. Similar results were obtained with other bicyclooctatrienes.

Conversion of **12a + b** to the mixed acetoxy derivatives, 3-acetamido-7-acetoxy-4- and 4-acetamido-7-acetoxy-3-nitrobicyclo[4.2.0]octa-1,3,5-triene (**13a + b**) was accomplished by

(12) Funk, R. L.; Vollhardt, K. P. C. *J. Chem. Soc., Chem. Commun.* **1976**, 833–4.

(13) Bunton, C. A.; Hughes, E. D.; Ingold, C. K.; Jacobs, D. I. H.; Jones, M. H.; Minkoff, G. J.; Reed, R. I. *J. Chem. Soc.* **1950**, 2628–56.

(14) (a) Horner, L.; Subramaniam, P. V.; Eiben, K. *Liebigs Ann. Chem.* **1968**, *714*, 91–111. (b) Schwartz, L. H.; Landis, J.; Lazarus, S. B.; Stoldt, S. H. *J. Org. Chem.* **1972**, *37*, 1979–84.

(15) Lloyd, J. B. F.; Ongley, P. A. *Tetrahedron* **1964**, *20*, 2185–94.

(16) Moder, K. P.; Duesler, E. N.; Leonard, N. J. *Acta. Crystallogr., Sect. B* **1981**, *B37*, 289–91.

using standard methodology.[17] Failure to maintain adequate agitation of the heterogeneous mixture containing silver acetate resulted in reduction in the yield of **13**. As postulated previously, the acetoxy derivative **13**, when subjected to raised temperature in the presence of dimethyl acetylenedicarboxylate, formed an *o*-xylylene intermediate with subsequent cycloaddition and elimination of acetic acid to give the tetra-β-substituted naphthalene, dimethyl 6-acetamido-7-nitro-2,3-naphthalenedicarboxylate (**14**), in good yield. The reaction sequence described above represents the first general methodology for the synthesis of tetra-β-varisubstituted naphthalenes. Tedious isolation procedures for **12** and **13** could be eliminated if the crude intermediates were used in subsequent reactions. Purification after formation of the naphthalene nucleus afforded **14** in good overall yield, along with recovered **11**, which could be recycled.

Deacetylation of **14** in refluxing methanolic HCl gave dimethyl 6-amino-7-nitro-2,3-naphthalenedicarboxylate (**15**) in excellent yield. Stringent exclusion of air during the reduction of the nitro group of **15** in methanol with 10% palladium-on-carbon was important, because dimethyl 6,7-diamino-2,3-naphthalenedicarboxylate (**16**) was readily oxidized in air. Extensive air oxidation, as characterized by darkening of the originally colorless diamine, resulted in a reduced isolable yield. Immediate acid-catalyzed ring closure with trimethyl orthoformate under an inert atmosphere gave dimethyl 1*H*-naphth[2,3-*d*]imidazole-6,7-dicarboxylate, which was isolated as the hydrochloride salt (**17**) in excellent yield.

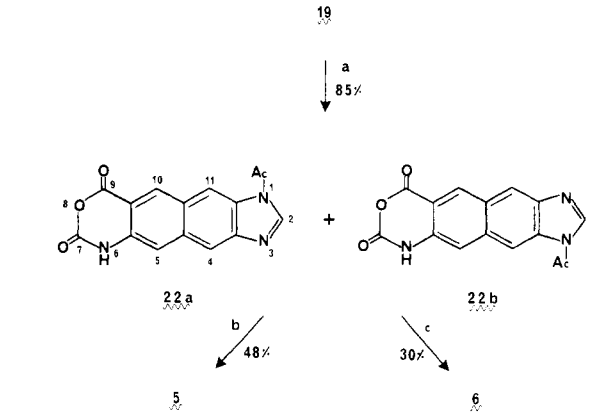
Base hydrolysis of **17** gave the diacid, 1*H*-naphth[2,3-*d*]-imidazole-6,7-dicarboxylic acid (**18**), which was not isolated because it was prone to decomposition. Instead, the crude diacid was dehydrated directly with acetic anhydride. Dehydration was accompanied by acetylation of the imidazole, giving 1-acetylnaphth[2,3-*d*]imidazole-6,7-dicarboxylic anhydride (**19**).

Two options were available for completion of the synthesis. First, the anhydride **19** could function as the starting material for the three-step conversion of aromatic anhydrides to pyrimidinediones.[18] The second option was conversion of **19** to an isatoic anhydride derivative which could be elaborated to a pyrimidine ring.[19] Both reaction routes were explored.

Stepwise conversion of anhydride **19** to the pyrimidinedione began with the synthesis of *N*-hydroxynaphth[2,3-*d*]imidazole-6,7-dicarboximide (**20**) in dry pyridine with hydroxylamine hydrochloride. The hydroxylamine generated in situ served both to convert the anhydride to the *N*-hydroxy dicarboximide and to deprotect the imidazole ring in a single step. Standard reaction conditions for the transformation of anhydrides to *N*-hydroxy dicarboximides, namely, hydroxylamine hydrochloride in refluxing pyridine, could not be employed because anhydride **19** decomposed readily above 80 °C in the presence of hydroxylamine. However, compound **20** was obtained in excellent yield by maintaining the temperature at 70–75 °C.

Tosylation of **20** proved difficult. Attempted tosylation of **20** with *p*-toluenesulfonyl chloride in dry pyridine gave incomplete reaction. Since **20** was nearly insoluble in refluxing pyridine, several attempts were made to tosylate the hydroxy imide in pyridine with dimethyl sulfoxide as a cosolvent. Addition of dimethyl sulfoxide gave increased solubility; however, the tosylation again was incomplete. It should be noted that *N*,*N*-dimethylformamide could not be used as a solent or cosolvent for this tosylation because of the addition product formed between *N*,*N*-dimethylformamide and *p*-toluenesulfonyl chloride.[20] The results described above appeared to indicate the failure to deprotonate

Scheme II[a]



[a] (a) $(CH_3)_3SiN_3$, py; (b) formamide; (c) urea.

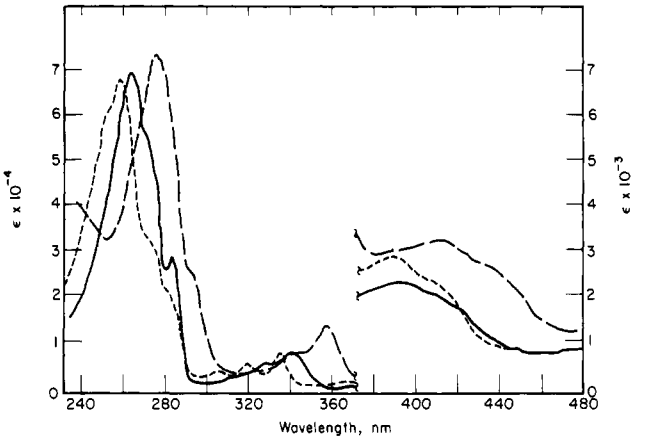


Figure 2. Ultraviolet absorption spectra of *lin*-naphthoxanthine (**6**) in 95% ethanol (—), 0.1 N HCl in 95% ethanol (- - -), and 0.1 N NaOH in 95% ethanol (– – –).

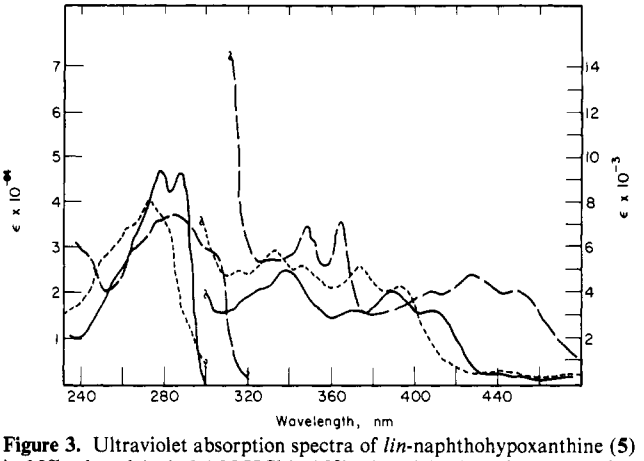


Figure 3. Ultraviolet absorption spectra of *lin*-naphthohypoxanthine (**5**) in 95% ethanol (—), 0.1 N HCl in 95% ethanol (- - -), and 0.1 N NaOH in 95% ethanol (– – –).

20. Addition of a stronger base, 4-(dimethylamino)pyridine, to the suspension of **20** in pyridine–dimethyl sulfoxide gave clean ditosylation. Several attempts to tosylate the *N*-hydroxy dicarboximide selectively were unsuccessful. To ensure complete ditosylation, excess 4-(dimethylamino)pyridine and *p*-toluenesulfonyl chloride were used in the reaction, which then furnished *N*-(*p*-tolylsulfonoxy)-1-*p*-tolylsulfonyl)naphth[2,3-*d*]imidazole-6,7-dicarboximide (**21**).

Final conversion to the pyrimidinedione, *lin*-naphthoxanthine, or benzimidazo[5,6-*g*]-6*H*,8*H*-quinazoline-7,9-dione (**6**), was accomplished by rearrangement of **21** to the partially deprotected *lin*-naphthoxanthine in ethanolic ammonia. Deprotection of the

(17) Nozaki, H.; Noyori, R.; Kôzaki, N. *Tetrahedron* **1969**, *20*, 641–5.
(18) (a) Bauer, L.; Miarka, S. V. *J. Am. Chem. Soc.* **1957**, *79*, 1983–5. (b) Kühle, E.; Wegler, R. *Liebigs Ann. Chem.* **1958**, *616*, 183–207. (c) Fahmy, A. F. M.; Aly, N. F.; Orabi, M. O. *Bull. Chem. Soc. Jpn.* **1978**, *51*, 2148–52. (d) VanVerst, M. E.; Bell, C. L.; Bauer, L. *J. Heterocycl. Chem.* **1979**, *16*, 1329–33.
(19) Coppola, G. M. *Synthesis* **1980**, 505–36.
(20) (a) Hall, H. K., Jr., *J. Am. Chem. Soc.* **1956**, *78*, 2717–27. (b) Albright, J. D.; Beng, E.; Lanzilotti, A. E.; Goldman, L. *J. Chem. Soc. Chem. Commun.* **1965**, 413–4.

Table I. Ultraviolet Absorption Spectra of *lin*-Naphthohypoxanthine (5) and *lin*-Naphthoxanthine (6)

compound	λ_{max} (95% EtOH), nm (log ϵ)
	λ_{max} (95% EtOH), nm (log ϵ)
lin-naphthohypoxanthine (5)	407 (3.58), 387 (3.69), 370 (3.58), 338 (3.71), 287 (4.69), 276 (4.69), 266 (sh) (4.53), 256 (sh) (4.35)
lin-naphthoxanthine (6)	390 (3.44), 339 (3.93), 325 (sh) (3.81), 282 (4.45), 271 (sh) (4.72), 263 (4.83)
	λ_{max} (0.1 N HCl) (95% EtOH), nm (log ϵ)
lin-naphthohypoxanthine (5)	391 (3.69), 372 (3.73), 344 (3.72), 332 (3.77), 292 (sh) (4.30), 280 (sh) (4.54), 272 (4.62), 262 (4.54)
lin-naphthoxanthine (6)	408 (sh) (3.43), 390 (3.55), 334 (3.93), 319 (3.78), 282 (sh) (4.30), 272 (sh), (4.48), 257 (4.80)
	λ_{max} (0.1 N NaOH) (95% EtOH), nm (log ϵ)
lin-naphthohypoxanthine (5)	447 (3.69), 426 (3.76), 407 (3.69), 363 (3.93), 346 (3.88), 304 (sh) (4.44), 284 (4.58), 278 (sh) (4.57), 264 (sh) (4.46)
lin-naphthoxanthine (6)	432 (sh) (3.50), 412 (3.62), 356 (4.21), 340 (sh) (4.01), 293 (sh) (4.40), 274 (4.84)

Table II. Fluorescence Spectral Data for *lin*-Naphthohypoxanthine (5) and *lin*-Naphthoxanthine (6)[a]

compound	solvent	excitation, nm (uncorr)	emission, nm (uncorr)	τ,[b] ns	ϕ[c]
lin-naphthohypoxanthine (5)	EtOH	325 (sh), 340, 373, 387, 410	460	24.8 ± 0.2[d]	0.70[d]
lin-naphthoxanthine (6)	EtOH	330 (sh), 343, 395	460	33.1 ± 0.5[e]	0.88[e]

[a] Purged of oxygen. [b] For fluorescence lifetime determinations, see Experimental Section. [c] Quantum yields, determined relative to quinine in 0.1 N H_2SO_4 (Φ = 0.70). [d] Excitation at 410 nm. [e] Excitation at 400 nm.

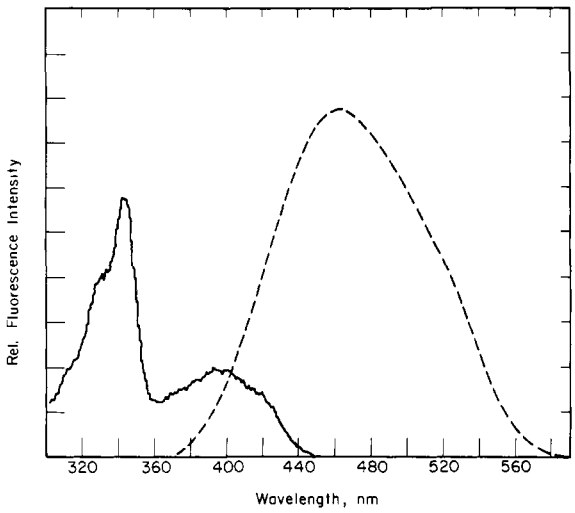


Figure 4. Technical fluorescence excitation (—) and emission (---) spectra of *lin*-naphthoxanthine (**6**) in ethanol purged of oxygen.

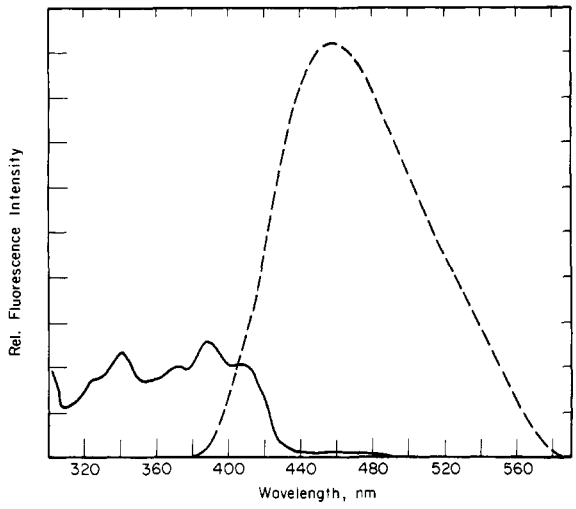


Figure 5. Technical fluorescence excitation (—) and emission (---) spectra of *lin*-naphthohypoxanthine (**5**) in ethanol purged of oxygen.

imidazole was completed by addition of aqueous base[21] to the reaction mixture in a second stage.

Unfortunately, compound **6** did not function efficiently as the desired versatile precursor for the other nucleic acid bases. The initial conversion reaction was to have been thiation of both carbonyls. Several attempts with purified phosphorus pentasulfide in dry pyridine[1f] or with Lawesson's reagent[22] in dry hexamethylphosphorus triamide or pyridine were unsuccessful; therefore, an alternative intermediate was sought. The isotoic anhydride route (Scheme II) held the advantage of potential synthesis of all four desired substituted tetracyclic compounds from a single intermediate without the need to proceed via a thiation reaction. Addition of trimethylsilyl azide[23] to a suspension of **19** in dry pyridine gave, after workup, 1- and 3-acetylbenzimidazo-[5,6-*g*]-8,6-benzoxazine-7,9(6*H*)-dione (**22a + b**).

Fusion of **22** with urea gave *lin*-naphthoxanthine **6**. The overall yield of **6** via the isatoic anhydride (30%) was lower than that obtained by the method described previously (50%). The lower yield via the isatoic anhydride route was due mainly to the inefficiency of the urea fusion (40%). Unlike compound **6**, isatoic anhydride **22** allowed access to other desired ring systems. *lin*-Naphthohypoxanthine, or benzimidazo[5,6-*g*]-8*H*-quinazolin-9-one (**5**), was formed by fusion of **22** with formamide.

The syntheses described above constitute the first report of linear nonheteroatom-substituted benzimidazoquinazolines. An all-oxazine derivative, imidazo[4,5-*i*]alloxazine, or benzimidazo-[5,6-*g*]-5,10-diazaquinazoline-7,9(6*H*,8*H*)-dione, was reported by Tul'chinskaya and co-workers.[24]

Optical Properties. The ultraviolet absorption spectra of *lin*-naphthoxanthine (Figure 2) and *lin*-naphthohypoxanthine (Figure 3) exhibit electronic absorption bands (Table I) over a broad range of wavelength. The *lin*-benzopurines displayed long-wavelength bands only in the 340-nm region; by contrast, the two *lin*-naphthopurines **5** and **6** showed absorption bands not only in the 340-nm region but also in the 400-nm region. The importance of the long-wavelength absorption bands will become apparent during the discussion of the fluorescence of **5** and **6**.

The importance of the series of dimensionally altered purines as sensitive biological probes would be seriously hampered if the analogues did not exhibit the spectroscopic property of fluorescence. Fluorescence techniques comprise a sensitive means of elucidating structural and dynamic properties of proteins, nucleic acids, and coenzymes.[2b,c,g-j,m,25] In contrast to the *lin*-benzopurines,

(21) Fujii, T.; Sakakibara, S. *Bull. Chem. Soc. Jpn.* **1974**, *47*, 3146–51.

(22) (a) Scheibye, S.; Pederson, B. S.; Lawesson, S. O. *Bull. Soc. Chim. Belg.* **1978**, *87*, 229–38. (b) Scheibye, S.; Pedersen, B. S.; Lawesson, S. O. *Ibid.* **1978**, *87*, 299–306. (c) Fritz, H.; Hug, P.; Lawesson, S. O.; Logemann, E.; Pedersen, B. S.; Sauter, H.; Scheibye, S.; Winkler, T. *Ibid.* **1978**, *87*, 525–34. (d) Clausen, K.; Lawesson, S. O. *Nouv. J. Chim.* **1980**, *4*, 43–6. (e) Shabana, R.; Scheibye, S.; Clausen, K.; Olesen, S. O.; Lawesson, S. O. *Ibid.* **1980**, *4*, 47–51.

(23) (a) Tsuge, O.; Urano, S.; Oe, K. *J. Org. Chem.* **1980**, *45*, 5130–6. (b) Groutas, W. C.; Felker, O. *Synthesis* **1980**, 861–8.

(24) Tul'chinskaya, L. D.; Zhilina, T. A.; Berzovskii, V. M. *Zh. Obshch. Khim.* **1974**, *44*, 406–10.

Table III. Kinetic Data[a] for Xanthine Oxidase

compound	K_m[b] (lit.)[c]	V_{max}[d]	K_i slope[b]
hypoxanthine	2.1×10^{-6} (1.3×10^{-6})	4.15×10^{-6}	[e]
lin-benzohypoxanthine	1.57×10^{-5}	1.78×10^{-5}	[f]
lin-naphthohypoxanthine (5)	1.01×10^{-5}	5.21×10^{-4}	3.04×10^{-6}
xanthine	1.1×10^{-6} (1.7×10^{-6})	5.36×10^{-6}	[e]
lin-naphthoxanthine (6)	[g]	[g]	4.03×10^{-6}

[a] Based on Lineweaver–Burk plots (least-squares linear fit to data). [b] In units of moles per liter. [c] Reference 26. [d] In units of moles per minute per milligram of protein. [e] Not applicable. [f] Not determined. [g] As a noncompetitive inhibitor, the measured $K_m = 1.0 \times 10^{-5}$ mol L^{-1} and $V_{max} = 1.62 \times 10^{-6}$ mol min^{-1} (mg of protein)$^{-1}$.

where fluorescence could not have been predicted a priori, the naphthalene series was expected to display good fluorescence characteristics. The naphthalene analogues are brilliantly fluorescent, as indicated by their notably high fluorescence quantum yields and lifetimes in 95% ethanol purged of oxygen (Table II). Since both the *lin*-benzopurines and the *lin*-naphthopurines exhibit long-wavelength absorption bands, well beyond the region of absorption of proteins and nucleic acids, these fluorophores can be selectively excited at long wavelengths in the presence of proteins and nucleic acids. The fluorescence emission and excitation spectra for *lin*-naphthoxanthine and *lin*-naphthohypoxanthine are shown in Figures 4 and 5. The fluorescence properties of the *lin*-naphthopurines, coupled with potential biological activity, complement those of the benzopurines which have demonstrated both favorable spectroscopic properties and enzyme activity.

Oxidation with Xanthine Oxidase. Xanthine oxidase is a complex enzyme which contains a hydrophobic pocket where oxidation takes place on the heteroaromatic portions of the nucleic acid base (e.g., hypoxanthine) as substrate. Inosine, the ribofuranosyl derivative of hypoxanthine, is not oxidized by xanthine oxidase, possibly due to unfavorable steric interaction as well as to the hydrophilic nature of the sugar moiety. For the *lin*-benzopurines, xanthine oxidase readily oxidizes *lin*-benzohypoxanthine to *lin*-benzoxanthine and thence to *lin*-benzouric acid,[1e] and *lin*-benzoinosine is also oxidized sequentially in both terminal rings.[1e] It is interesting to note that *lin*-benzoinosine 5′-monophosphate is oxidizable to the corresponding *lin*-benzoxanthosine 5′-monophosphate without subsequent oxidation.[2k] These results suggest that the lateral displacement of the ribosyl or ribosyl 5′-phosphate moiety from the pyrimidine ring permitted oxidation at C-6 (corresponding to C-2 in the purine ring system), and only the ribosyl 5′-phosphate moiety obstructed oxidation at C-2.

Synthesis of *lin*-naphthoxanthine (**6**) and *lin*-naphthohypoxanthine (**5**) allowed us to examine further the dimensional tolerance of the binding pocket in xanthine oxidase. Attempted oxidation of **6** with xanthine oxidase, using a modified assay method with oxygen as the final electron acceptor, was unsuccessful (Table III). This result, while not unexpected, provided the first example of a linear base analogue incapable of functioning as a substrate for xanthine oxidase. Previously, *dist*-benzoxanthine (Figure 6, **a**) was the only extended base analogue not converted to the corresponding uric acid derivative.[1e] However, the cause for interruption of the oxidation at the xanthine stage could not be identified definitively as resulting from steric interference of the imidazole ring or hydrogen bonding between N-1 and N–H at the 9 position. Interestingly, *prox*-benzoxanthine (Figure 6, **b**) could be oxidized by xanthine oxidase to the uric acid derivative,

Figure 6.

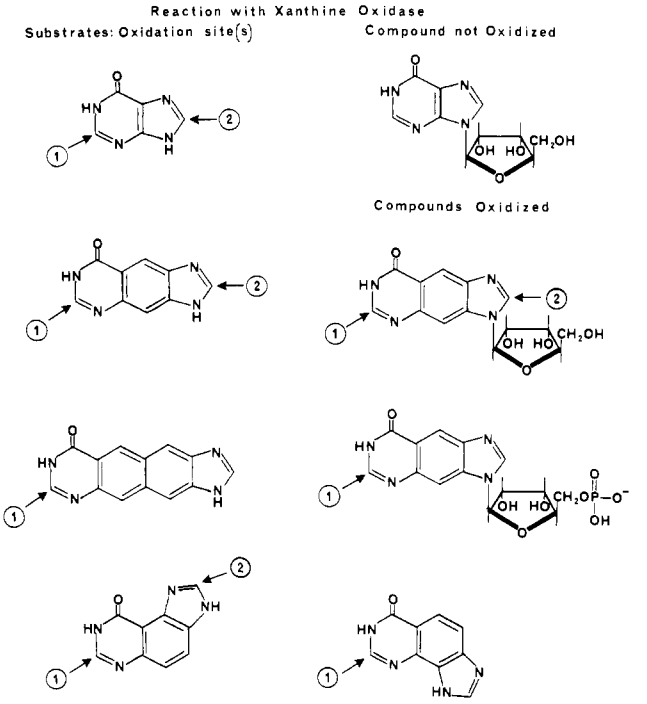


Figure 7.

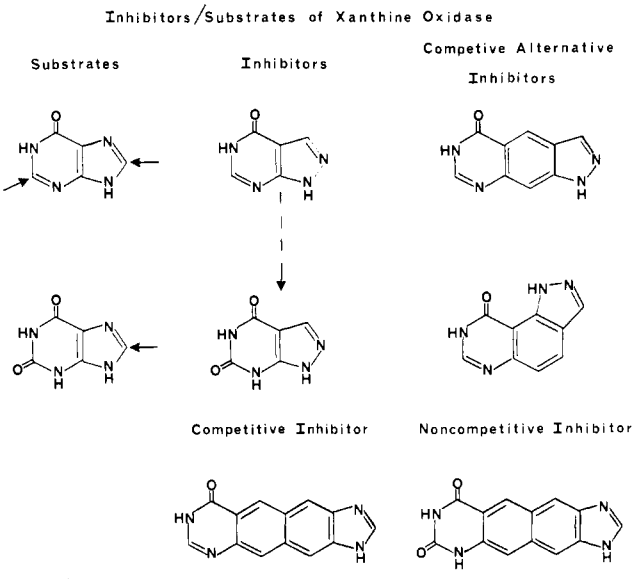


Figure 8.

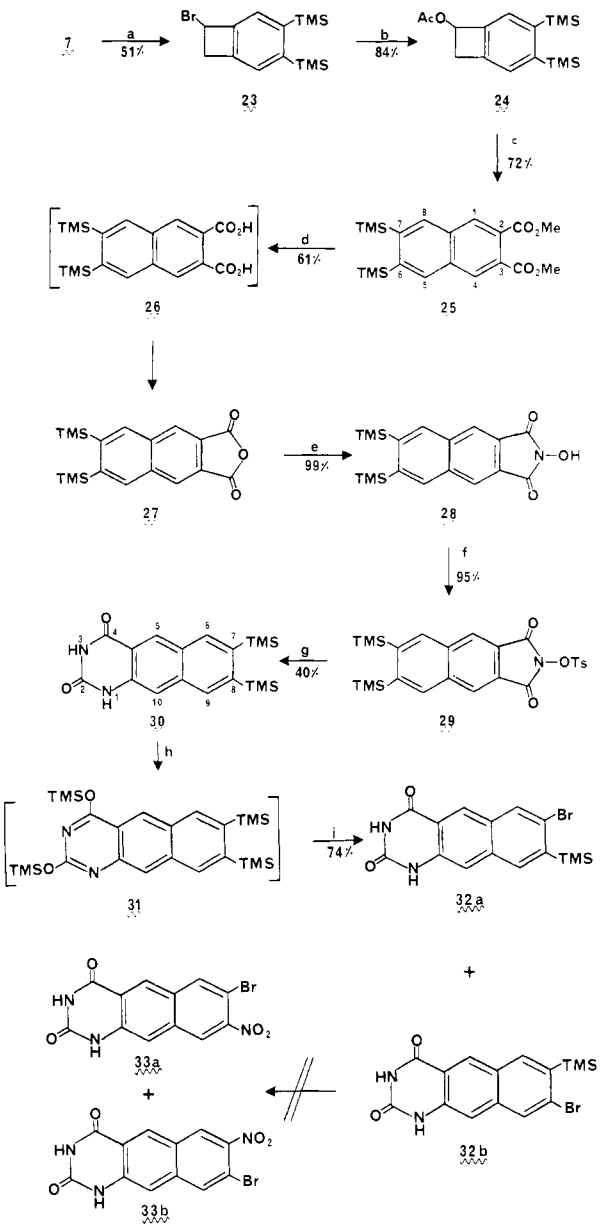
but at a much slower rate than the xanthine to uric acid conversion.[1e] The shape of this molecule does not provide steric interference except in relation to the rate of oxidation.

The possibility whether the *lin*-naphthoxanthine could function as an inhibitor of oxidation of xanthine to uric acid by xanthine oxidase was explored. The apparent Michaelis constants and relative velocities, K_m and V_{max}, for *lin*-naphthoxanthine and xanthine, similarly measured, were found to be nearly identical (Table III) and in the range of values reported for xanthine.[26]

(25) (a) Leonard, N. J.; Tolman, G. L. *Ann. N.Y. Acad. Sci.* **1975**, *255*, 43–58. (b) Stryer, L. *Science* **1968**, *162*, 526–33. (c) Udenfriend, S. "Fluorescence Assay in Biology and Medicine"; Academic Press: New York, 1962; Vol. 1. (d) Udenfriend, S. "Fluorescence Assay in Biology and Medicine"; Academic Press: New York, 1969; Vol. II. (e) Cantor, C. R.; Tao, T. "Procedures in Nucleic Acid Research"; Cantoni, G. L., Davies, D. R., Eds.; Harper and Row: New York, 1971; Vol. II.

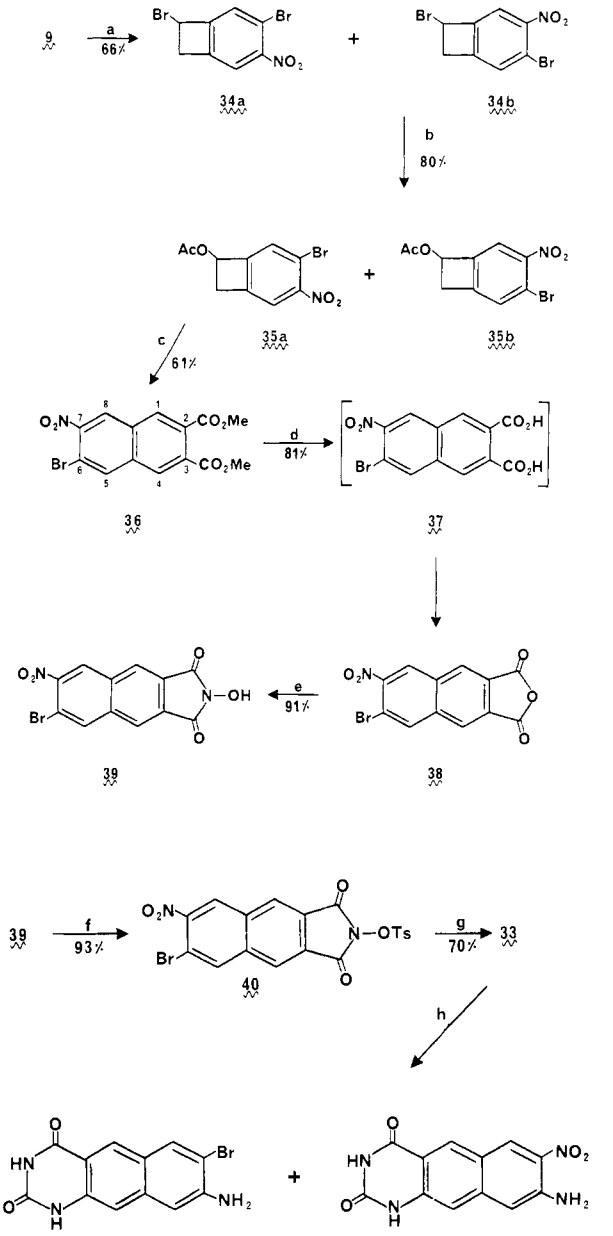
(26) "Biochemica Information"; Boehringer Mannheim GmbH: Mannheim, West Germany, 1973; Vol. 1, p 179.

Scheme III[a]



[a] (a) NBS, CCl_4, $(PhCO_2)_2$; (b) AgOAc, HOAc; (c) DMAD, st; (d) NaOH, MeOH; H_3O^+; Ac_2O (e) $H_2NOH{\cdot}HCl$, py; (f) TsCl, py; (g) NH_3, EtOH; (h) $(CH_3)_3SiCl$, HMDS; (i) Br_2, CCl_4, py.

Scheme IV[a]



[a] (a) NBS, CCl_4, *hv*, $(PHCO_2)_2$; (b) AgOAc, HOAc; (c) DMAD, st; (d) NaOH, MeOH; H_3O^+; AcCl; (e) $H_2NOH{\cdot}HCl$, py; (f) TsCl, py; (g) NH_3, EtOH; (h) NH_3, *n*-BuOH, bomb.

The ultraviolet absorption spectrum for the oxidation product of *lin*-naphthohypoxanthine (**5**) by xanthine oxidase and air was identical with an authentic sample of *lin*-naphthoxanthine (**6**). The V_{max} values increase in the order hypoxanthine < *lin*-benzohypoxanthine < *lin*-naphthohypoxanthine (Table III). The K_m values for *lin*-benzohypoxanthine and *lin*-naphthohypoxanthine are similar. *lin*-Naphthohypoxanthine functions as a competitive inhibitor of the oxidation of hypoxanthine by xanthine oxidase, and *lin*-naphthoxanthine (prepared via Scheme II), as shown by double-reciprocal Lineweaver–Burk plots, functions as a noncompetitive inhibitor.

We have achieved, in part, our ultimate goal of setting limits on spatial restrictions of an enzyme binding region. The initial enzymatic investigations with the naphthalene analogues, when coupled with the data obtained from the benzene analogue series, clearly indicate substrate structure restrictions for xanthine oxidase. The ability of buttermilk xanthine oxidase to produce *lin*-benzouric acids and not *lin*-naphthouric acid requires that the binding pocket at an oxidation site on or within the enzyme be unable to accommodate the larger lateral extension of the naphthalene derivatives to accomplish oxidation in the imidazole ring. Therefore, one can tentatively assign the limiting lateral extension of the substrate for xanthine oxidase to be able to act on the imidazole ring to be between 2.4 and 4.8 Å, corresponding to the benzo and naphtho analogues, respectively. The results are summarized in Figures 7 and 8, in which are also included comparative results for allopurinol and two benzoallopurinols, *lin* and *prox*.[1h,2l] Additional research is in progress to develop derivatives that refine the limiting restrictions.

Subsidiary Synthetic Pathways. The reaction sequences presented above were based on experience gained while exploring less fruitful routes. Two such routes are presented in Schemes III and IV. Each reaction sequence merits discussion because it demonstrates the versatility of the synthetic methodology employed to construct tetra-β-substituted naphthalenes.

In Scheme III, the trimethylsilyl groups were allowed to remain intact until the latter stages of the reaction sequence. Problems were then encountered during the attempted formation of the

imidazole ring precursor. The first pyrimidinedione intermediate, 7,8-bis(trimethylsilyl)benzo[5,6-*g*]-1*H*,3*H*-quinazoline-2,4-dione (**30**), was insoluble in carbon tetrachloride, which generally serves as the standard bromodesilylation solvent. However, the bis(trimethylsilyl) ether, 2,3-bis(trimethylsiloxy)-7,8-bis(trimethylsilyl)benzo[5,6-*g*]quinazoline (**31**), was freely soluble in the carbon tetrachloride, but then bromodesilylation resulted in a mixture of isomers, 7-bromo-8- and 8-bromo-7-(trimethylsilyl)benzo[5,6-*g*]-1*H*,3*H*-quinazoline-2,4-dione (**32a + b**).

Completion of the synthesis was envisioned to follow the sequence of nitrodesilylation, nucleophilic aromatic displacement of the aryl bromide with ammonia, reduction, and ring closure to give **6**. Attempted nitrodesilylation of **32** resulted in decomposition and formation of an oxidized product which was a quinone derivative of **32**, as revealed by mass spectral analysis, but of undetermined oxidation positions. This result can be explained by examining the mechanism for nitrodesilylation and assuming that **32** behaved as a typical naphthalene derivative. Nitrodesilylation can proceed via nitrosodesilylation followed by oxidation to the nitro compound.[27] However, naphthalene itself is oxidized by CrO_3 at 0 °C to 1,4-naphthoquinone[28] under conditions similar to those used to convert nitroso groups to nitro groups.[29] One can perceive that, under the strong oxidizing reaction conditions employed, **32** could readily have been converted to a quinone derivative. Therefore, it was recognized as necessary to introduce the functionality for the imidazole ring precursor at a point preceding the formation of the naphthalene ring. Scheme IV illustrates how the aforementioned problems were surmounted.

Introduction of the bromo and nitro groups prior to construction of the naphthalene ring system was successful (Scheme IV). However, an unanticipated difficulty was encountered during attempted nucleophilic displacement of the mixture of aryl bromides, 7-bromo-8- and 8-bromo-7-nitrobenzo[5,6-*g*]-1*H*,3*H*-quinazoline-2,4-dione (**33a + b**), with ammonia which resulted in decomposition and the formation of multiple products under a variety of reaction conditions. The components of the product mixtures were determined by mass spectral analysis. The main product from the nucleophilic displacement reaction was 8-amino-7-bromobenzo[5,6-*g*]-1*H*,3*H*-quinazoline-2,4-dione (**41**). Compound **41** arose from the nucleophilic displacement of the nitro group in **33a**. Although displacement of multiactivated nitro groups is more familiar,[30] monoactivated nucleophilic displacements are known.[31] In our case, the nitro group was preferentially activated over the bromo, through the naphthalene ring system, by the carbonyl at C-4 of the pyrimidinedione ring. Therefore, one can conclude that, of the two possible bromonitrobenzoquinazoline isomers **33a** and **33b**, **33a** must predominate in the synthesis outlined in Scheme IV.

The probable cause for the predominant formation of the less desirable isomer **33a**, which gave rise to the incorrect amine **41**, can be rationalized on the basis of comparison of the mechanism of a similar type of rearrangement that provided the pyrimidinedione **33**. In the Hoffman rearrangement,[32] the carbonyl with the lower electron density, which is the carbonyl para to the nitro group, is attacked by the incoming nucleophile. The result is then retention of that carbonyl group para to the nitro function. In our system, the nitro group would decrease the electron density at the carbonyl carbon in the amphi position, on C-3 (see Scheme IV), thereby affording **33a** as the predominant rearrangement product.

The subsidiary pathways served to emphasize the importance of preconstruction of the imidazole ring for the successful formation of the complete heterocyclic system and to provide further samplings of tetra-β-substituted naphthalenes that have become available by the general synthetic methodology described.

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Experimental Section

General Comments. All thin-layer chromatographic separations were performed on Merck precoated silica gel f-254 plates with fluorescent backing. Melting points were determined on a Büchi melting point apparatus and are uncorrected. The NMR spectra were recorded on a Varian Associates EM-390 or HA-220 spectrometer using tetramethylsilane as an internal standard. Mass spectra were obtained by Mr. J. Carter Cook and his staff on a Varian MAT CH-5 low-resolution mass spectrometer or a Varian MAT-331 low-resolution field desorption mass spectrometer coupled with a 620i computer and a Statos recorder. Ultraviolet absorption spectra were obtained on a Beckman Acta MVI spectrometer. Technical fluorescence excitation and emission spectra were obtained on a Spex Fluorolog spectrofluorometer. Fluorescence quantum yields and lifetimes were determined by Dr. D. M. Jameson. The pressure vessels used were purchased from Parr Instrument Co. Microanalyses were performed by Mr. Josef Nemeth and his staff or by Midwest Microlab, Ltd. Buttermilk xanthine oxidase (xanthine:oxygen oxidoreductase; EC No. 1.2.3.2), grade III, was purchased from Sigma Chemical Co.

3,4-Bis(trimethylsilyl)bicyclo[4.2.0]octa-1,3,5-triene (7). The reaction was a modification of the original Vollhardt[6] method. All manipulations were performed under an argon atmosphere until the chromatographic purification. To a round-bottomed flask equipped with a magnetic stirring bar, reflux condenser, and two septum caps was added bis(trimethylsilyl)acetylene (80 mL). Argon was passed through the solvent for 0.5 h. To the solvent was added $CpCo(CO)_2$ (0.3 mL). In separate gastight syringes were added 1,5-hexadiyne (8.0 g, 0.102 mmol) and a solution of $CpCo(CO)_2$ (0.5 mL) in bis(trimethylsilyl)acetylene (9.5 mL). The vigorously stirred reaction mixture was heated in a 140 °C oil bath. The contents of the syringes were added over 60 h by means of a syringe pump. The reaction was cooled and the excess solvent was vacuum distilled. The dark oily residue was chromatographed on a column (3 cm × 80 cm) of silica gel (300) in pentane. The homogeneity of the fraction was assayed by TLC in pentane. Removal of the solvent gave a yellow oil which crystallized from ether–methanol at −20 °C as colorless orthorhombic crystals and was air-dried for 2 h (6.3 g, 25%); mp 43 °C (lit.[6] mp 43–44 °C).

3-Bromo-4-(trimethylsilyl)bicyclo[4.2.0]octa-1,3,5-triene (8). Vollhardt's procedure[6] was used to synthesize **8**. A solution of Br_2 (10.2 g, 72 mmol) in CCl_4 (20 mL) was added with vigorous stirring to an ice-cold solution of **7** (9.0 g, 36 mmol) in CCl_4 (70 mL) and pyridine (2.7 mL). The mixture was stirred at 0 °C for 1 h. the reaction mixture was diluted with ether (250 mL) and extracted with saturated aqueous $Na_2S_2O_3$ (150 mL) and then H_2O (75 mL). The organic layer was dried over Na_2SO_4. After filtration and solvent removal, the light yellow oil was crystallized from ether–methanol at −78 °C as a colorless powder. The product was air-dried for 24 h (8.0 g, 88%); mp 54–55 °C (lit.[6] mp 55–56 °C). The compound was stored in a dark bottle at 2 °C.

3-Bromo-4-nitrobicyclo[4.2.0]octa-1,3,5-triene (9). Caution: This reaction should be carried out in an efficient fume hood. The nitrodesilylation solution was prepared by slow addition of nitric acid (3.6 mL) to ice-cold acetic anhydride (18 mL). This solution was added slowly by means of a syringe pump to a vigorously stirred solution of **8** (6.0 g, 23.6 mmol) in glacial acetic acid (36 mL) which was heated in a 95–100 °C oil bath for 2.5 h. After the addition was complete, the mixture was cooled and poured into ice-cold aqueous 1.0 N NaOH (200 mL). The resulting yellow suspension was extracted with ether (3 × 200 mL). The combined ether extracts were dried over $MgSO_4$, filtered, and then concentrated to afford an orange-brown oil. The oil was swirled in pentane (20 mL). Upon cooling, a yellow-brown precipitate was formed. The precipitate was triturated repeatedly with hot pentane until the pentane washes were colorless. The combined pentane washes were cooled to −78 °C, which caused a yellow solid to separate. The solid was collected and air-dried for 12 h (3.7 g, 69%). This material was used without further purifications. However, bright yellow analytical crystals could be obtained by several recrystallizations from pentane: mp 68–69 °C; mass spectrum, 70 eV, *m/e* (rel intensity) 229 (55, M + 2), 227 (56, M^+), 171 (29), 169 (30), 102 (100); ^1H NMR (CCl_4) δ 3.27 (s, 4, $(CH_2)_2$), 7.35 (s, 1, Ar H), 7.40 (s, 1, Ar H).

Anal. Calcd for $C_8H_6BrNO_2$: C, 42.14; H, 2.65; Br, 35.04; N, 6.14. Found: C, 42.39; H, 2.55; Br, 35.04; N, 6.22.

3-Amino-4-nitrobicyclo[4.2.0]octa-1,3,5-triene (10). Compound **9** (4.0 g, 17.6 mmol) was placed in a 300-mL bomb equipped with a magnetic stirring bar. An ammonia solution was made by passing anhydrous NH_3(g) through 1-butanol (150 mL) at −78 °C for 1 h. This solution (210 mL) was added to the bomb, which had been cooled to −78 °C, and

the bomb was closed. After being heated with stirring at 170–175 °C in an oil bath for 4 days, the bomb was recooled to −78 °C and opened carefully. The excess ammonia was removed under a stream of nitrogen while the reaction mixture warmed to room temperature. Removal of the solvent gave a dark oily residue. This residue was dissolved in hot 1:1 (v/v) ethanol–water (50 mL), treated with decolorizing carbon, and filtered. The filtrate was diluted with H_2O (50 mL) and cooled to −20 °C for 3 h. Crystallization was completed with the addition of more H_2O (100 mL). The solid was collected and air-dried for 24 h, which afforded 2.2 g (76%); mp 108 °C (lit.[33] mp 110 °C).

3-Acetamido-4-nitrobicyclo[4.2.0]octa-1,3,5-triene (11). A suspension of **10** (5.0 g, 30.1 mmol) in acetyl chloride (50 mL) was heated to reflux on a steam bath for 10 min. The reaction mixture was filtered hot. The solvent was removed and the yellow-brown solid was triturated with hot ether until the ether washes were colorless. The ethereal solutions were combined and concentrated until the solution became turbid. The solution was slowly cooled to −20 °C. The yellow needles were collected and air-dried for 12 h (4.0 g, 65%); mp 97 °C (lit.[33] mp 97–98 °C).

3-Acetamido-7-bromo-4- and 4-Acetamido-7-bromo-3-nitrobicyclo-[4.2.0]octa-1,3,5-triene (12a + b). Recrystallized NBS (1.8 g, 9.7 mmol) and benzoyl peroxide (spatula tip) were added to a solution of **11** (2.0 g, 9.7 mmol) in CCl_4 (100 mL). The mixture was brought to reflux simultaneously with irradiation with a 250-W flood lamp for 3 h. The reaction mixture was cooled and filtered. Concentration of the solution gave an orange-brown oil. The crude bromide was used without further purification.

3-Acetamido-7-acetoxy-4- and 4-Acetamido-7-acetoxy-3-nitrobicyclo-[4.2.0]octa-1,3,5-triene (13a + b). Silver acetate (2 g) was added to a solution of crude bromide **12** in glacial acetic acid (20 mL). The mixture was stirred vigorously and heated in a 110–115 °C oil bath for 24 h. After the reaction mixture was cooled and filtered, the solvent was removed. The brown oil was treated with ether and the mixture was filtered. The solid was triturated with hot ether until the washes were colorless. Removal of the solvent gave crude **13** as an orange-brown oil. The crude product **13** was used without further purification; ^{1}H NMR (CCl_4) δ 2.03 (s, 3, CH_3), 2.20 (s, 3, CH_3), 3.24 (dd, 1, CH), 2.67 (dd, 1, CH), 5.77 (dd, 1, CHOAc), 7.98 (s, 1, Ar H), 8.6 (s, 1, Ar H), 10.53 (br, 1, NH).

Dimethyl 6-Acetamido-7-nitro-2,3-naphthalenedicarboxylate (14). A solution of crude **13** in dimethyl acetylenedicarboxylate (2 mL) was placed in a sealed tube and heated in a 150–155 °C oil bath for 24 h. The reaction mixture was cooled and the dark oily residue was dissolved in $CHCl_3$. The diluted reaction mixture was adsorbed onto silica gel (5 g). The adsorbed material was dry-loaded on a column (3 cm × 40 cm) of silica gel (300 g) in 1:1 (v/v) pentane–ether. The column was eluted with 1:1 (v/v) pentane–ether until product began to appear. The homogeneity of the fractions was assayed on TLC in 1:1 (v/v) pentane–ether. The solvent was then changed to ether and elution was continued until the product eluted completely from the column. The product fractions were combined and concentrated to a small volume (~20 mL). Upon concentration, the product precipitated from the solution as a yellow solid. It was collected and washed with ice-cold ether (20 mL) and air-dried for 24 h: yield 0.74 g (32%); mp 174 °C; yield (based upon unreacted **11**) 0.6 g, 2.92 mmol; mass spectrum, 10 eV, *m/e* (rel intensity) 346 (56, M^+), 304 (100), 300 (19), 273 (13); ^{1}H NMR ($(CD_3)_2SO$) δ 2.43 (s, 3, $COCH_3$), 3.87 (s, 6, CO_2CH_3), 8.20 (s, 1, Ar H), 8.60 (s, 1, Ar H), 8.83 (s, 1, Ar H), 10.53 (br, 1, NH).

Anal. Calcd for $C_{16}H_{14}N_2O_7$: C, 55.50; H, 4.08; N, 8.09. Found: C, 55.70; H, 4.21; N, 8.25.

Dimethyl 6-Amino-7-nitro-2,3-naphthalenedicarboxylate (15). A suspension of **14** (0.7 g, 2 mmol) in methanolic HCl (100 mL) was heated at reflux on a steam bath for 2.5 h. The reaction mixture was cooled, and removal of solvent gave a yellow solid, which was recrystallized from methanol as red plates (0.55 g, 91%): mp 177 °C; mass spectrum, 10 eV, *m/e* (rel intensity) 304 (100, M^+), 273 (68); ^{1}H NMR ($(CD_3)_2SO$) δ 3.83 (s, 6, CO_2CH_3), 7.07 (br, 2, NH_2), 7.37 (s, 1, Ar H), 7.87 (s, 1, Ar H), 8.42 (s, 1, Ar H), 8.92 (s, 1, Ar H).

Anal. Calcd for $C_{14}H_{12}N_2O_6$: C, 55.27; H, 3.98; N, 9.21. Found: C, 55.10; H, 3.84; N, 8.96.

Dimethyl 6,7-Diamino-2,3-naphthalenedicarboxylate (16). In small portions, 10% Pd/C was carefully added to a suspension of **15** (0.5 g, 1.6 mmol) in methanol (100 mL). The mixture was hydrogenated for 2 h. The system was flushed several times with argon, and the reaction mixture was filtered under argon. Removal of solvent gave a colorless to light yellow solid **16**. The crude diamine was used without purification.

Dimethyl 1*H*-Naphth[2,3-*d*]imidazole-6,7-dicarboxylate Hydrochloride (17). The crude diamine **16** was suspended in trimethyl orthoformate (30 mL) and 98–100% formic acid (5 drops). The mixture was brought to reflux under argon for 3 h. The solution was cooled, and removal of solvent gave a yellow oil, which was dissolved in a minimum amount of methanol. The methanolic solution was cooled to 0 °C and was saturated with anhydrous HCl(g). The cream-colored precipitate was collected and washed with ice-cold methanol (5 mL) and anhydrous ether (20 mL). The product was air-dried for 24 h (0.515 g, 99%): mp >300 °C; mass spectrum, 10 eV, *m/e* (rel intensity) 284 (100, M^+ − HCl), 253 (60); ^{1}H NMR ($(CD_3)_2SO$) δ 3.22 (s, 6, CO_2CH_3), 6.57 (br, 1, NH), 8.63 (s, 4, Ar H), 9.83 (s, 1, Ar H).

Anal. Calcd for $C_{15}H_{13}ClN_2O_4$: C, 56.17; H, 4.09; N, 8.73; Cl, 11.05. Found: C, 55.96; H, 4.08; 8.75; Cl, 11.01.

1*H*-Naphth[2,3-*d*]imidazole-6,7-dicarboxylic Acid (18). A solution of **17** (0.3 g, 0.94 mmol) in methanol (3 mL) and 2 N NaOH (3 mL) was heated on a steam bath for 0.5 h. The reaction mixture was cooled and acidified with aqueous 2 N HCl (5 mL). Upon acidification, a colorless precipitate formed. Removal of the solvent gave a colorless residue. Crude **18** was used without further purification.

1-Acetylnaphth[2,3-*d*]imidazole-6,7-carboxylic Anhydride (19). Compound **18** was suspended in acetic anhydride (30 mL) and the mixture was heated at reflux for 1 h. The hot reaction mixture was filtered to remove the salt, and the filtrate was concentrated to dryness. The colorless residue **19** was recrystallized from acetic anhydride at −20 °C and was dried at 80 °C (0.05 mmHg) for 12 h (0.203 g, 77%): mp 280 °C dec; mass spectrum, 10 eV, *m/e* (rel intensity) 280 (75, M^+) 238 (100), 194 (22); ^{1}H NMR ($(CD_3)_2SO$) δ 2.78 (s, 3, $COCH_3$), 8.25 (s, 1, Ar H), 8.42 (s, 1, Ar H), 8.47 (s, 1, Ar H), 8.65 (s, 1, Ar h), 8.72 (s, 1, Ar H).

Anal. Calcd for $C_{15}H_8N_2O_4$: C, 64.29; H, 2.88; N, 10.00. Found: C, 63.96; H, 2.74; N, 9.96.

1*H*-*N*-Hydroxynaphth[2,3-*d*]imidazole-6,7-dicarboximide (20). Hydroxylamine hydrochloride (0.6) was added to a suspension of **19** (0.3 g, 1.1 mmol) in dry pyridine (6 mL). The mixture was stirred with heating in an 80 °C oil bath for 12 h with the exclusion of moisture. The components dissolved upon heating, and after 0.5 h a yellow precipitate began to form. After cooling, the reaction mixture was poured onto water (50 mL). The yellow precipitate was collected and washed with water (50 mL), 0.01 N HCl (20 mL), and again with H_2O (50 mL). An analytical sample was obtained by recrystallization from acetic acid–water. Compound **20** was dried at 80 °C (0.05 mmHg) for 12 h (0.27 g, 96%); mp 300 °C; mass spectrum, 10 eV, *m/e* (rel intensity) 253 (25, M^+), 237 (82); ^{1}H NMR ($(CD_3)_2SO$) δ 3.93 (br, OH), 8.62 (s, 2, Ar H), 8.65 (s, 2, Ar H), 9.20 (s, 1, Ar H).

Anal. Calcd for $C_{13}H_7N_3O_3$: C, 61.66; H, 2.79; N, 16.60. Found: C, 61.55; H, 2.89; N, 16.44.

***N*-*p*-(Tolylsulfonoxy)-1-*p*-(tolylsulfonyl)naphth[2,3-*d*]imidazole-6,7-dicarboximide (21).** Dimethyl-4-aminopyridine (0.4 g, 3.3 mmol) was added to a vigorously stirred suspension of **20** (0.2 g, 0.8 mmol) in pyridine (5 mL) and $(CH_3)_2SO$ (15 mL). The mixture was stirred for 5–10 min; then purified *p*-tolunesulfonyl chloride (0.5 g, 2.6 mmol) was added. After the yellow solution was stirred for 1.5 h, it was poured into H_2O (60 mL). The precipitate was collected and washed with H_2O (100 mL), 0.01 N HCl (50 mL), and again with H_2O (100 mL). Compound **21** was air-dried overnight and then at 80 °C (0.05 mmHg) for 12 h (0.32 g, 72%): mp 200 °C dec; mass spectrum (field desorption), *m/e* 561 (M^+); ^{1}H NMR ($(CD_3)_2SO$) δ 2.33 (s, 3, $Ar_{C7'}$ CH_3), 2.53 (s, 3, $Ar_{C7''}$ CH_3), 7.45 (d, 2, $Ar_{C3',5'}$ H), 7.53 (d, 2, $Ar_{C2',6'}$ H), 7.97 (d, 2, $Ar_{C3'',5''}$ H), 8.18 (d, 2, $Ar_{C2'',6''}$ H), 8.70 (s, 2, Ar H), 8.87 (s, 2, Ar H), 9.18 (s, 1, Ar H).

Anal. Calcd for $C_{27}H_{19}N_3O_7S_2$: C, 57.75; H, 3.41; N, 7.48; S, 11.42. Found: C, 57.78; H, 3.38; N, 7.74; S, 11.37.

Benzimidazo[5,6-*g*]-6*H*,8*H*-quinazoline-7,9-dione (6). Method A. A suspension of **21** (0.3 g, 0.535 mmol) in ethanolic ammonia (15 mL) was heated at reflux for 1 h. The reaction mixture was cooled to 25 °C and aqueous 2.0 N NaOH (10 mL) was added. The resulting solution was stirred at 25 °C for 1 h. The pH of the reaction mixture was adjusted carefully to 9.0 with concentrated hydrochloric acid, which resulted in the formation of a yellow precipitate. The reaction mixture was absorbed onto silica gel (2 g) and then dry-loaded on a column (3 cm × 40 cm) of silica gel (250 g) in 4:1 (v/v) $CHCl_3$–MeOH. When the product began to elute, the solvent system was changed to 3:2 (v/v) $CHCl_3$–MeOH. The homogeneity of the fractions was assayed by TLC on silica gel with 4:1 (v/v) $CHCl_3$–MeOH as solvent. The appropriate fractions were combined and the volume reduced to about 25 mL. Upon concentration, a yellow solid formed. The precipitate was collected and washed with hot H_2O (20 mL), MeOH (20 mL), and ether (20 mL). The yellow solid was dried at 80 °C (0.05 mmHg) for 12 h, which afforded 0.12 g (84%): mp >300 °C; mass spectrum, 10 eV, *m/e* (rel intensity) 252 (100, M^+), 208 (47); ^{1}H NMR ($(CD_3)_2SO$) δ 7.37 (s, 1, Ar H), 7.80 (s, 1, Ar H), 8.13 (s, 1, Ar H), 8.30 (s, 1, Ar H), 8.57 (s, 1, Ar H), 10.93 (s, 1, NH), 11.00 (s, 1, NH).

Anal. Calcd for $C_{13}H_8N_4O_2 \cdot {}^1/_2H_2O$: C, 59.77; H, 3.47; N, 21.45.

(33) Lloyd, J. B. F.; Ongley, P. A. *Tetrahedron* **1965**, *21*, 2281–8.

Found: C, 59.72; H, 3.34, N, 21.10.

Method B. An intimate mixture of **22** (20 mg, 0.068 mmol) and urea (100 mg, 1.7 mmol) was heated in a 185 °C oil bath for 1 h. Water (5 mL) was added carefully to the hot reaction mixture. The resulting suspension was poured into boiling H_2O (300 mL) and boiled for 0.5 h. The solution was filtered hot and then reduced in volume (25 mL). After the solution was cooled overnight at 0 °C, a yellow solid was collected and dried at 80 °C (0.05 mmHg) for 12 h, which gave 6.0 mg (30%).

1- and 3-Acetylbenzimidazo[5,6-*g*]-8,6-benzoxazine-7,9(6*H*)-dione (22a + b). Trimethylsilyl azide (2 mL) was added to a suspension of **19** (0.2 g, 0.71 mmol) in dry pyridine (6.0 mL). The reaction mixture was stirred at 25 °C for 1 h with the exclusion of moisture. However, when the solid did not dissolve after 15 min, the reaction mixture was warmed to 35 °C for a few minutes and then cooled back down to 25 °C for the rest of the reaction time. The solvent volume was concentrated (1 mL) under reduced pressure at 25 °C. Upon addition of *n*-hexene (50 mL), a flocculent yellow precipitate formed. The precipitate was collected and washed with *n*-hexane (30 mL). The solid was dried at 80 °C (0.05 mmHg) for 12 h, which afforded 0.18 g (85%): mp >300 °C; mass spectrum, 10 eV, *m/e* (rel intensity) 295 (55, M^+), 253 (54), 251 (100, $M^+ - CO_2$), 209 (99), 44 (59); ^1H NMR ($(CD_3)_2SO$) δ 3.23 (s, 3, $COCH_3$), 8.57 (m, 5, Ar H).

Anal. Calcd for $C_{15}H_9N_3O_4$: C, 61.0; H, 3.05; N, 14.24. Found: C, 61.46; H, 3.05; N, 14.06.

Benzimidzo[5,6-*g*]-8*H*-quinazolin-9-one (5). A suspension of **22** (75 mg, 0.25 mmol) in formamide (3 mL) was heated with stirring in a 180 °C oil bath for 1 h. Water (5 mL) was added cautiously to the hot reaction mixture. The precipitate was collected and suspended in methanol (10 mL). The methanolic suspension was absorbed onto silica gel (1 g) and then dry-loaded on a column (120 g) of silica gel (2 cm × 40 cm) in 9:1 (v/v) $CHCl_3$–MeOH. The homogeneity of the fractions was assayed by TLC on silica gel with 9:1 (v/v) $CHCl_3$–MeOH as the solvent. The appropriate fractions were collected and concentrated (5 mL). This solution was added to boiling water (250 mL) and then boiled for 0.5 h. After filtration of the hot solution, the filtrate was reduced in volume (50 mL) and cooled at 0 °C overnight. The product was collected and dried at 80 °C (0.05 mmHg) for 12 h, which furnished 28 mg (48%): mp >300 °C; mass spectrum, 10 eV, *m/e* (rel intensity) 236 (100, M^+); ^1H NMR ($(CD_3)_2SO$) δ 7.42 (s, 1, Ar H), 7.46 (s, 1, Ar H), 8.00 (s, 1, Ar H), 8.02 (s, 1, Ar H), 8.36 (s, 1, Ar H), 8.98 (s, 1, Ar H).

Anal. Calcd for $C_{13}H_8N_4O$: C, 66.10; H, 3.41; N, 23.72. Found: C, 66.47; H, 3.47; N, 23.53.

7-Bromo-3,4-bis(trimethylsilyl)bicyclo[4.2.0]octa-1,3,5-triene (23). To a solution of **7** (1.0 g, 4 mmol) in CCl_4 (60 mL) were added recrystallized NBS (0.73 g, 4 mmol) and a few grains of benzoyl peroxide. The mixture was heated at reflux for 1 h. The reaction mixture was cooled to 25 °C and filtered, and the filtrate was concentrated to an orange oil. The oil was chromatographed on a column (3 cm × 80 cm) of silica gel (300 g) in pentane. The homogeneity of the fractions was assayed by TLC in pentane. After the appropriate fractions had been combined, removal of the solvent afforded a colorless oil (0.66 g, 51%): mass spectrum, 10 eV, *m/e* (rel intensity) 328 (58, M + 2), 326 (60, M^+), 313 (100), 311 (94), 297 (31), 295 (29), 247 (58), 159 (49); ^1H NMR (CCl_4) δ 0.33 (s, 18, $SiCH_3$), 3.38 (dd, 1, C_8H_a, ^2J = 15 Hz, ^3J = 3 Hz), 3.81 (dd, 1, C_8H_b, ^2J = 15 Hz, ^3J = 6 Hz), 5.25 (dd, 1, C_7H), 7.27 (s, 1, Ar H) 7.30 (s, 1, Ar H).

Anal. Calcd for $C_{14}H_{23}BrSi_2$: C, 51.36; H, 7.08; Br, 24.41. Found: C, 51.65; H, 7.15; Br, 24.67.

7-Acetoxy-3,4-bis(trimethylsilyl)bicyclo[4.2.0]octa-1,3,5-triene (24). A solution of glacial acetic acid (4 mL) and acetic anhydride (1 mL) was heated at reflux for 1 h with exclusion of moisture. To the anhydrous acetic acid was added **23** (0.351 g, 2.1 mmol) and silver acetate (0.4 g, 2.4 mmol). The reaction mixture was stirred vigorously and heated in a 100–110 °C oil bath for 6 h and then cooled to room temperature and filtered. The solid residue was washed with acetic acid (20 mL), and the filtrate was concentrated to an orange oil. The oil was chromatographed on a column (3 cm × 40 cm) of silica gel (150 g) with a 1-L gradient of pentane to 9:1 (v/v) pentane–ether. The homogeneity of the fractions was assayed by TLC in 9:1 (v/v) pentane–ether. The appropriate fractions were combined, and solvent removal afforded a yellow oil (0.54 g, 84%): mass spectrum, 10 eV, *m/e* (rel intensity) 306 (42, M^+), 291 (28), 264 (60), 249 (100), 246 (97); ^1H NMR (CCl_4) δ 0.33 (s, 18, $SiCH_3$), 1.98 (s, 3, $COCH_3$), 3.07 (dd, 1, C_8H_a, ^2J = 15 Hz, ^3J = 3 Hz), 3.55 (dd, 1, C_8H_b, ^2J = 15 Hz, ^3J = 6 Hz), 5.70 (dd, 1, C_7H), 7.28 (s, 1, Ar H), 7.33 (s, 1, Ar H).

Anal. Calcd for $C_{16}H_{26}O_2Si_2$: C, 62.69; H, 8.55. Found: C, 62.60; H, 8.51.

Dimethyl 6,7-Bis(trimethylsilyl)-2,3-naphthalenedicarboxylate (25). To a solution of **24** (3.6 g, 11.8 mmol) in dry xylene (3 mL) was added dimethyl acetylenedicarboxylate (3 mL). The resulting solution was placed in a sealed tube and was flushed with argon for 0.5 h and then heated in a 150–155 °C oil bath for 12 h. The reaction mixture was cooled, and the dark oily mixture was chromatographed on a column (3 cm × 40 cm) of silica gel (150 g) with a 1-L gradient of pentane to 9:1 (v/v) pentane–ether. The homogeneity of the fractions was assayed by TLC in 9:1 (v/v) pentane–ether. Removal of the solvent from the combined fractions afforded **24** (1.3 g, 4.3 mmol) and the desired product. The product was recrystallized from methanol at −78 °C and dried at 25 °C (0.05 mmHg) for 12 h (2.1 g, 72% based on unrecovered starting material): mp 88–90 °C; mass spectrum, 10 eV, *m/e* (rel intensity) 388 (100, M^+), 372 (44); ^1H NMR ($(CD_3)_2SO$) δ 0.53 (d, 18, $SiCH_3$), 3.90 (s, 6, CO_2CH_3), 8.37 (m, 4, Ar H).

Anal. Calcd for $C_{20}H_{28}OSi_2$: C, 61.82; H, 7.26. Found: C, 61.67; H, 7.20.

6,7-Bis(trimethylsilyl)-2,3-naphthalenedicarboxylic Acid (26). To a solution of **25** (1.2 g, 3.1 mmol) in methanol (10 mL) was added 2 N NaOH (10 mL). The mixture was heated at reflux for 24 h. The reaction mixture was then cooled and acidified with 2 N HCl. The aqueous suspension was extracted with ether (4 × 30 mL), and the combined ether extracts were dried over Na_2SO_4. The ethereal solution was filtered. Removal of the solvent gave the crude diacid as a colorless residue, which was used without further purification.

6,7-Bis(trimethylsilyl)-2,3-naphthalenedicarboxylic Anhydride (27). The solid residue **26** was suspended in acetic anhydride (30 mL) and the mixture was heated at reflux for 5 h. The reaction mixture was cooled to −20 °C overnight. The solid was collected and recrystallized as colorless needles from chloroform–heptane. The solid **27** was dried at 80 °C (0.05 mmHg) for 12 h (0.62 g, 61%): mp 230 °C; mass spectrum, 10 eV, *m/e* (rel intensity) 342 (100, M^+), 327 (48); ^1H NMR ($(CD_3)_2SO$) δ 0.48 (s, 18, $SiCH_3$), 8.30–8.76 (m, 4, Ar H).

Anal. Calcd for $C_{18}H_{22}O_3Si_2$: C, 63.12; H, 6.47. Found: C, 62.89; H, 6.53.

***N*-Hydroxy-6,7-bis(trimethylsilyl)-2,3-naphthalenedicarboximide (28).** Hydroxylamine hydrochloride (1.6 g) was added to a solution of **27** (0.83 g, 2.4 mmol) in dry pyridine (30 mL). The solution was stirred and heated in a 80 °C oil bath for 5 h. The reaction mixture was cooled and poured onto ice–H_2O (400 mL). The light tan precipitate was collected, washed with H_2O (100 mL), 0.05 N HCl (100 mL), and again with H_2O (100 mL), and was recrystallized from dioxane–pentane as colorless needles. The product was air-dried at 80 °C (0.05 mmHg) for 12 h (0.86 g, 99%): mp 266–230 °C dec; mass spectrum, 10 eV, *m/e* (rel intensity) 357 (42, M^+), 342 (100), 326 (58); ^1H NMR ($(CD_3)_2SO$) δ 0.43 (s, 18, $SiCH_3$), 3.35 (br, 1, NOH), 8.40 (4, Ar H).

Anal. Calcd for $C_{18}H_{23}O_3Si_2$: C, 60.47; H, 6.48; N, 3.93. Found: C, 60.53; H, 6.59; N, 3.99.

***N*-*p*-(Tolylsulfonoxy)-6,7-bis(trimethylsilyl)-2,3-naphthalenedicarboximide (29).** Recrystallized *p*-toluenesulfonyl chloride (1.6 g, 8.4 mmol) was added to a solution of **28** (0.86 g, 2.4 mmol) in dry pyridine (15 mL). After the mixture was stirred for 0.5 h at 25 °C, it was poured onto ice–H_2O (200 mL). The light yellow solid was collected and washed with H_2O (200 mL), 0.05 N HCl (100 mL), and again with H_2O (100 mL) and then dried at 80 °C (0.05 mmHg) for 12 h (1.2 g, 95%): mp 185 °C; mass spectrum (field desorption), *m/e* 511 (M^+); ^1H NMR ($(CD_3)_2SO$) δ 0.47 (s, 18, $SiCH_3$), 3.33 (s, 3, Ar CH_3), 7.52 (d, 2, Ar_{T_s} H), 7.97 (d, 2, Ar_{T_s} H), 8.22 (m, 4, Ar H).

Anal. Calcd for $C_{25}H_{29}NO_5SSi_2$: C, 57.92; H, 5.47; N, 2.81; S, 6.44. Found: C, 58.08; H, 5.52; N, 2.54; S, 6.46.

7,8-Bis(trimethylsilyl)benzo[5,6-*g*]-1*H*,3*H*-quinazoline-2,4-dione (30). A suspension of **29** (0.87 g, 1.7 mmol) in saturated ethanolic ammonia at −20 °C was heated at reflux for 3 h. The reaction mixture was cooled and poured onto H_2O (200 mL). The precipitate was collected and air-dried for 24 h. The solid was recrystallized from acetic acid as a colorless solid, which was then dried at 140 °C (0.05 mmHg) for 12 h (0.24 g, 40%): mp >300 °C; mass spectrum, 10 eV, *m/e* (rel intensity) 356 (100, M^+), 341 (67), 325 (32); ^1H NMR ($(CD_3)_2SO$) δ 0.37 (s, 18, $SiCH_3$), 7.60 (s, 1, ArH), 8.22 (s, 1, Ar H), 8.45 (s, 1, Ar H), 8.78 (s, 1, Ar H).

Anal. Calcd for $C_{18}H_{24}N_2O_2Si_2$: C, 60.63; H, 6.78; N, 7.86. Found: C, 60.48; H, 6.79; N, 7.69.

7,8-Bis(trimethylsilyl)-2,4-bis[(trimethylsilyl)oxy]benzo[5,6-*g*]-quinazoline (31). A suspension of **30** (166 mg, 0.45 mmol) in hexamethyldisilazane (6 mL) and trimethylsilyl chloride (4 mL) was heated under dry nitrogen at reflux with vigrous stirring for 12 h. The reaction mixture was cooled and the solvent was removed under reduced pressure. The crude material was used without purification.

7-Bromo-8- and 8-Bromo-7-(trimethylsilyl)benzo[5,6-*g*]-1*H*,3*H*-quinazoline-2,4-dione (32a + b). To a solution of crude **31** in dry CCl_4 (1 mL) was added dry pyridine (40 μL) and a solution of Br_2 (50 μL) in dry CCl_4 (0.5 mL). The resulting suspension was stirred at 0 °C for 3 h. The reaction mixture was diluted with anhydrous ether (20 mL) and

extracted with saturated aqueous $Na_2S_2O_3$. The organic layer was washed with H_2O (20 mL) and dried over Na_2SO_4. The dried solution was filtered, and removal of solvent gave **32a + b** (0.124 g, 74%): mp >300 °C; mass spectrum, 10 eV, *m/e* (rel intensity) 364 (100, M + 2), 362 (97, M^+), 349 (58), 347 (53), 267 (58); ^1H NMR ($(CD_3)_2SO$) δ 0.37 (s, 9, $SiCH_3$), 7.63 (d, 1, Ar H), 7.97 (d, 1, Ar H), 8.40 (d, 1, Ar H), 8.69 (d, 1, Ar H).

Anal. Calcd for $C_{15}H_{15}BrN_2O_2Si$: C, 49.59; H, 4.16; Br, 22.00; N, 7.71. Found: C, 49.36; H, 4.10; Br, 21.98; N, 7.50.

3,7-Dibromo-4- and 4,7-Dibromo-3-nitrobicyclo[4.2.0]octa-1,3,5-triene (34a + b). Recrystallized NBS (0.5 g, 2.94 mmol) and a few grains of benzoyl peroxide were added to a solution of **9** (0.6 g, 2.64 mmol) in CCl_4 (32 mL). The reaction was heated at reflux and was irradiated with a 250-W flood lamp for 12 h. The reaction mixture was cooled and filtered. Concentration of the filtrate gave an orange oil, which was chromatographed on a column (3 cm × 80 cm) of silica gel (300 g) in 9:1 (v/v) pentane–ether. The homogeneity of the fractions was assayed by TLC in 9:1 (v/v) pentane–ether. Combination of the appropriate fractions gave **9** (0.3 g, 1.32 mmol) and **34** as a yellow oil (0.26 g, 66%, based on unreacted starting material): mass spectrum of **34a + b**: 10 eV, *m/e* (rel intensity) 309 (24, M + 4), 307 (41, M + 2), 305 (23, M^+), 228 (88), 226 (100); ^1H NMR (CCl_4) δ 3.05–3.85 (m, 2, CH_2), 5.07 (m, 1, CH), 7.08 (s, 1, Ar H), 7.17 (d, 1, Ar H).

Anal. Calcd for $C_8H_5Br_2NO_2$: C, 31.30; H, 1.64; N, 4.56; Br, 52.07. Found: C, 30.99; H, 1.70; N, 4.26; Br, 51.90.

After concentration, **34a + b** was used in the next reaction without further purification.

7-Acetoxy-3-bromo-4- and 7-Acetoxy-4-bromo-3-nitrobicyclo[4.2.0]-octa-1,3,5-triene (35a + b). Silver acetate (0.5 g) was added to a solution of crude **34a + b** in dry glacial acetic acid (3 mL). The mixture was stirred vigorously while being heated in a 110 °C oil bath. After 24 h, an additional 0.5 g of silver acetate was added to the reaction mixture. After an additional 24 h, the reaction mixture was cooled, filtered, and concentrated to afford a yellow-orange oil. The oil was chromatographed on a column (3 cm × 40 cm) of silica gel (150 g) with a 1-L gradient of 9:1 (v/v) pentane–ether to 1:1 (v/v) pentane–ether. The homogeneity of the fractions was assayed by TLC in 9:1 (v/v) pentane–ether and the appropriate fractions were combined. Solvent removal gave **9** (0.21 g, 0.93 mmol) and **35** as a yellow oil (0.29 g, 80%): mass spectrum, 10 eV, *m/e* (rel intensity) 287 (1, M + 2), 285 (2, M^+), 243 (50), 241 (52), 43 (100); ^1H NMR (CCl_4) δ 1.97 (s, 3, $COCH_3$), 3.0–3.85 (m, 2, CH_2), 5.48 (m, 1, CH), 7.1 (s, 1, Ar H), 7.2 (s, 1, Ar H).

Anal. Calcd for $C_{10}H_8BrNO_4$: C, 41.98; H, 2.82; N, 4.90; Br, 27.93. Found: C, 41.97; H, 2.86; N, 4.62; Br, 28.02.

Dimethyl 6-Bromo-7-nitro-2,3-naphthalenedicarboxylate (36). Dimethyl acetylenedicarboxylate (3.5 mL) and a solution of **35a + b** (3.7 g, 13 mmol) in dry xylene (3 mL) were placed in a sealed tube and heated in a 150 °C oil bath for 24 h. Upon cooling, **36** crystallized from the reaction mixture. The yellow solid was washed with ice-cold xylene and was dried at 80 °C (0.05 mmHg) for 24 h (2.9 g, 61%): mp 180–181 °C; mass spectrum, 10 eV, *m/e* (rel intensity) 369 (58, M + 2), 367 (49, M^+), 338 (44), 336 (59), 259 (51), 257 (50), 245 (33), 243 (37), 230 (91), 228 (100); ^1H NMR ($(CD_3)_2SO$) 4.03 (s, 6, CO_2CH_3), 8.70 (s, 1, Ar H), 8.83 (s, 1, Ar H), 9.00 (s, 1, Ar H), 9.18 (s, 1, Ar H).

Anal. Calcd for $C_{14}H_{10}BrNO_6$: C, 45.68; H, 2.74; N, 3.81; Br, 21.71. Found: C, 45.70; H, 2.68; N, 3.77; Br 21.93.

6-Bromo-7-nitro-2,3-naphthalenedicarboxylic Acid (37). A suspension of **36** (0.5 g, 1.36 mmol) in methanol (13 mL) and 2 N NaOH (13 mL) was heated at reflux on a steam bath for 2 h. The reaction was cooled to 25 °C and acidified slowly with 2 N HCl (15 mL). The colorless suspension was extracted with ether (4 × 50 mL). The combined ether extracts were dried over Na_2SO_4. The solution was filtered and evaporated, and the crude diacid was used without further purification.

6-Bromo-7-nitro-2,3-naphthalenedicarboxylic Anhydride (38). The residue **37** was suspended in acetyl chloride (100 mL), brought to reflux, and kept there for 5 h. The reaction mixture was filtered hot and then evaporated. The solid was recrystallized from glacial acetic acid as yellow needles (0.35 g, 81%): mp 290 °C dec; mass spectrum, 10 eV, *m/e* (rel intensity) 323 (100, M + 2), 321 (98, M^+), 279 (88), 277 (92); ^1H NMR ($(CD_3)_2SO$) δ 9.00 (s, 1, Ar H), 9.10 (s, 2, Ar H), 9.25 (s, 1, Ar H).

Anal. Calcd for $C_{12}H_4BrNO_5$: C, 44.75; H, 1.25; N, 4.35; Br, 24.81. Found: C, 44.74; H, 1.03; N, 4.38; Br, 24.87.

***N*-Hydroxy-6-bromo-7-nitro-2,3-naphthalenedicarboximide (39).** Hydroxylamine hydrochloride (0.2 g) was added to a suspension of **38** (0.2 g, 0.62 mmol) in dry pyridine (5 mL). The mixture was heated with stirring in an 80 °C oil bath with the exclusion of moisture for 7 h. The reaction solution was cooled to room temperature and poured onto ice–H_2O (60 mL). The brown precipitate was collected and washed with H_2O (50 mL), 0.5 N HCl (50 mL), and again with H_2O (50 mL). The resulting cream-colored solid was dried at 80 °C (0.05 mmHg) for 12 h, which gave 0.189 g (91%): mp 252 °C dec; mass spectrum, 10 eV, *m/e* (rel intensity) 338 (86, M + 2), 336 (100, M^+), 322 (47), 320 (43); ^1H NMR ($(CD_3)_2SO$) δ 3.43 (br, 1, NOH), 8.72 (s, 1, Ar H), 8.80 (s, 1, Ar H), 9.00 (s, 1, Ar H), 9.18 (s, 1, Ar H).

Anal. Calcd for $C_{12}H_5BrN_2O_5$: C, 42.76; H, 1.50; N, 8.31; Br, 23.70. Found: C, 42.84; H, 1.44; N, 8.38; Br, 23.82.

***N*-(*p*-Tolylsulfonoxy)-6-bromo-7-nitro-2,3-naphthalenedicarboximide (40).** Recrystallized *p*-toluenesulfonyl chloride (0.057 g, 0.3 mmol) was added to a stirred solution of **39** (0.1 g, 0.3 mmol) in dry pyridine (2 mL), and stirring was continued at 25 °C for 0.5 h. The reaction mixture was poured onto ice–H_2O (40 mL). The cream-colored solid was collected and washed with H_2O (50 mL), 0.1 N HCl (50 mL), and again with H_2O (50 mL) and then dried at 80 °C (0.05 mmHg) for 24 h, which afforded 0.14 g (93%): mp 250 °C dec; ^1H NMR ($(CD_3)_2SO$) δ 2.88 (s, Ar CH_3), 7.73 (d, 2, Ar_{T_s} H), 8.18 (d, 2, Ar_{T_s} H), 8.85 (s, 1, Ar H), 8.95 (s, 1, Ar H), 9.05 (s, 1, Ar H), 9.20 (s, 1 Ar H).

Anal. Calcd for $C_{19}H_{11}BrN_2O_7S$: C, 46.45; H, 2.26; N, 5.70; Br, 16.27; S, 6.53. Found: C, 46.49; H, 2.13; N, 5.74; Br, 16.54; S, 6.27.

7-Bromo-8- and 8-Bromo-7-nitrobenzo[5,6-*g*]-1*H*,3*H*-quinazoline-2,4-dione (33a + b). A suspension of **40** (0.5 g, 1.02 mmol) in saturated ethanolic ammonia (20 mL) was heated at reflux for 2 h. The reaction mixture was cooled to 0 °C, and a yellow solid was collected, washed with ethanol (20 mL), 2.0 N HCl (20 mL), and recrystallized from glacial acetic acid, and dried at 140 °C (0.05 mmHg) for 24 h, which gave 0.24 g (70%): mp 300 °C; mass spectrum, 10 eV, *m/e* (rel intensity) 337 (91, M + 2), 335 (100, M^+), 294 (20), 292 (18); ^1H NMR ($(CD_3)_2SO$) δ 3.38 (s, 2, NH), 7.80 (s, 1, Ar H), 7.92 (s, 1, Ar H), 8.75 (s, 1, Ar H), 8.92 (s, 1, Ar H), 8.95 (s, 1, Ar H), 8.98 (s, 1, Ar H), 9.08 (s, 1, Ar H), 9.17 (s, 1, Ar H).

Anal. Calcd for $C_{12}H_6BrN_3O_4$: C, 42.88; H, 1.80; N, 12.50; Br, 23.78. Found: C, 42.88; H, 1.77; N, 12.44; Br, 23.87.

Formation of 7-Bromo-8-aminobenzo[5,6-*g*]-1*H*,3*H*-quinazoline-2,4-dione (41) and 8-Amino-7-nitrobenzo[5,6-*g*]-1*H*,3*H*-quinazoline-2,4-dione (42). A suspension of **33** (100 mg, 0.3 mmol) is saturated 1-butanolic ammonia (60 mL) was placed in a 125-mL pressure vessel. The pressure vessel was sealed and heated in a 140 °C oil bath for 24 h. The vessel was cooled to −78 °C and opened carefully. Excess ammonia was removed under a stream of nitrogen. Removal of solvent gave a dark solid, which was analyzed by mass spectrometry to consist of **41** (10%) and **42** (2%), thus indicating that this route was less satisfactory than that described earlier in the Experimental Section.

Fluorescence. The technical fluorescence emission and excitation spectra obtained at 25 °C were not corrected for monochromator efficiency and photomultiplier response. The absolute quantum yields of compounds **5** and **6** were determined by comparison with the fluorescence emission of quinine sulfate (quantum yield 0.70 in 0.1 N H_2SO_4),[34] in solutions purged of oxygen (argon was passed through each solution at a rate of 1 bubble per 2 s for 30 min).

Fluorescence lifetimes were determined at 25 °C in solutions purged of oxygen using the cross-correlation spectrofluorometer described by Spencer and Weber.[35] The exciting light was modulated at 18 MHz and was filtered through a monochromator, a CS-3-72 Corning filter, and a sodium nitrite filter. The emission was observed through a CS-0-52 Corning filter. Fluorescence lifetime determinations of **5** and **6** by both phase and modulation were identical using quinine sulfate as a standard.

Oxidation with Xanthine Oxidase and Oxygen. The procedures used were modifications of the method of xanthine oxidase assay described by Boehringer Mannheim.[26] Final assay mixtures had a total volume of 3.0 mL in a cuvette with a 1.0-cm light path. The assay mixtures contained oxygen as the final electron acceptor,[21] Na_2EDTA at 10 mM, potassium phosphate buffer, pH 7.6 at 0.1 M, and the substrate to be oxidized. The substrate solutions were prepared by dissolving the sample in a minimum amount of $(CH_3)_2SO$ and then adding this solution to hot phosphate buffer.[21] The final concentrations of the substrate solution were as follows: xanthine, 6.55×10^{-1} mM; hypoxanthine, 3.64×10^{-1} mM; *lin*-naphthoxanthine (**6**), 9.13×10^{-2} mM; and *lin*-naphthohypoxanthine (**5**), 4.92×10^{-2} mM. The assay mixtures contained from 50 to 150 μL of each substrate solution with increases of 25-μL increments. Each assay was initiated by addition of 5 μL of an enzyme solution which was a dilution of Sigma buttermilk xanthine oxidase, grade III (7 mg/mL suspension in 2.3 M ammonium sulfate, 10 mM sodium phosphate buffer, pH 7.8, containing 1 mM EDTA and 1 mM sodium salicylate), in 0.1 M postassium phosphate, pH 7.6, in a ratio of 1:1 for xanthine and

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hypoxanthine, 1:9 for *lin*-naphthoxanthine, and 1:3 for *lin*-naphthohypoxanthine. The oxidation for xanthine and hypoxanthine was monitored at 293 nm, while the oxidation for *lin*-naphthoxanthine and *lin*-naphthopoxanthine was monitored at 260 and 267 nm, respectively. Duplicate assays were run for each sample.

In a study of the possible inhibitory effect of *lin*-naphthoxanthine and *lin*-naphthohypoxanthine on uric acid formation from xanthine and hypoxanthine, duplicate samples were run at each of the concentrations given above for xanthine and hypoxanthine at known inhibitor concentrations. Inhibitor concentrations were varied from 8.2×10^{-4} to 2.5×10^{-3} mM for *lin*-naphthoxanthine and 1.52×10^{-3} to 4.57×10^{-3} mM for *lin*-naphthohypoxanthine. Final assay mixtures had a total volume of 3 mL in a cuvette with a 1.0-cm light path. Each assay was initiated by addition of 5 μL of the Sigma buttermilk xanthine oxidase solution, which was a dilution in a ratio of 1:1 in phosphate buffer. The assays were monitored at 293 nm.

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Foreshortened Nucleotide Analogues as Potential Base-Pairing Complements for *lin*-Benzoadenosine

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Abstract: Syntheses of foreshortened nucleotide analogues of uridine have been carried out to test the possibility of base pairing with the linearly extended nucleoside *lin*-benzoadenosine. Phosphorylation of *N*-(β-D-ribofuranosyl)formamide (F) provided the 5-monophosphate, which could be dephosphorylated by the action of either alkaline phosphatase or, surprisingly, 5′-nucleotidase. Additional phosphorylations by the method of Hoard and Ott afforded the 5-di- and triphosphates. The diphosphate, 5-FDP, was found not to undergo polymerization with polynucleotide phosphorylase. Syntheses of the self-complementary dinucleoside monophosphates FpA and Fp(*lin*-benzo-A) are described. The foreshortened analogue was protected as its 2-(methoxytetrahydropyranyl)-5-(*tert*-butyldiphenylsilyl) derivative, while 5′-AMP and *lin*-benzo-AMP were protected by a new and easy method as the corresponding 2′,3′-di-*O*-(*tert*-butyldimethylsilyl) nucleotides. Condensation of the fully protected F and 5′-monophosphate moieties with DCC provided the desired (3→5′)-linked nucleotides, which, on treatment with phosphodiesterase I, were hydrolyzed back to F and the corresponding 5′-monophosphate.

Introduction

The complementarity of base pairing in the hydrogen-bonded associations of guanine with cytosine and adenine with thymine or uracil has provided the structural basis for much of molecular biology. It has now been almost 30 years since Watson and Crick recognized the two salient features of this scheme: first, that good linear hydrogen bonds can be formed in these matches, and second (and more importantly), that the same geometry accommodates A–T(U), T(U)–A, G–C, and C–G within the interior of a helix formed by two complementary strands of polymeric DNA or RNA.[1,2] While alternative base-pairing schemes are known to exist,[3] the same stringent geometrical constraints are imposed and must be satisfied in order for a stable duplex to exist.

In conjunction with our continuing investigations concerning the synthesis and utility of dimensional probes for the study of enzyme–coenzyme binding sites,[4] we have been interested in examining the possibility of base pairing with the linearly extended benzonucleotides. *lin*-Benzoadenine nucleotides (e.g., **4**, R = phosphoribosyl) are defined by the formal insertion of a benzene ring (actually four carbon atoms) into the center of the adenine ring system (**2**) and consequently are 2.4 Å wider than adenine. In a base-pairing scheme analogous to that of A–U, one might envision that *lin*-benzoadenosine could hydrogen bond to uridine or ribothymidine, but a double helix made up of stretched cross sections consisting of tricyclic plus monocyclic bases would be distorted from the normal. By contrast, a perfectly proportioned double helix can be constructed with an acyclic, or "zero-ring", complementary partner. The ideal complement would, by ne-

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(2) Cantor, C. R.; Schimmel, P. R. "Biophysical Chemistry"; W. H. Freeman: San Francisco, CA, 1980; Part 1, p 177.
(3) Reference 2, pp 319–321.
(4) For example, see: Leonard, N. J. *Heterocycles* **1979**, *12*, 129.

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