Synthesis of *lin*-Benzoinosine, *lin*-Benzoxanthosine, and *lin*-Benzoguanosine

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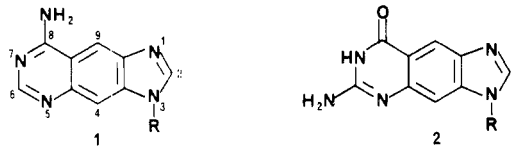
Syntheses of *lin*-benzoinosine, *lin*-benzoxanthosine, and *lin*-benzoguanosine (substituted 3-ribosylimidazo-[4,5-*g*]quinazolin-8-ones) are reported. Ribosidation of the mercuric salt of 6-(ethylthio)imidazo[4,5-*g*]-quinazolin-8-one gave a common intermediate in which the ethylthio group was displaced by ammonia to give *lin*-benzoguanosine or was reductively removed to give *lin*-benzoinosine. *lin*-Benzoinosine was oxidized by xanthine oxidase to give *lin*-benzoxanthosine.

Investigations utilizing 3-β-D-ribofuranosyl-8-amino-imidazo[4,5-*g*]quinazoline (**1b**)[1] (*lin*-benzoadenosine)[2] and



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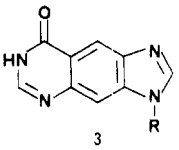

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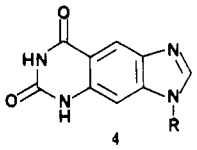

4

a, R = H

b, R = β-D-ribofuranose

c, R = β-D-ribofuranosyl 5'-phosphate

its phosphate derivatives[3,4] have shown that they are effective coenzymes for a variety of enzyme reactions.[3] The fluorescence properties,[5-8] the 2.4-Å lateral extension of the purine nucleus, and the unaltered loci of interaction make this system uniquely analogous to adenosine and its phosphate derivatives. We have previously reported the synthesis of *lin*-benzoguanine (**2a**)[9] and *lin*-benzohypoxanthine (**3a**),[10] the latter a precursor of **1**. We report here[11] the preparation of *lin*-benzoguanosine (**2b**), *lin*-benzoinosine (**3b**), and *lin*-benzoxanthosine (**4b**) by a divergent synthetic pathway. This pathway and an alternative one, also included, make available a wide variety of ribosylpurine analogues for discerning the role of purine in protein and nucleic acid synthesis, metabolic regulation, and enzyme–coenzyme interactions.

Results and Discussion

The keys to preparation of laterally extended tricyclic systems analogous to purine ribosides are the introduction of a carbon and three nitrogens in a 1,2,4,5 pattern on a central benzene nucleus, with little or no regioisomer formation, and unequivocal introduction of the ribofuranosyl moiety at the desired position in a common intermediate. These critical path determinants were solved as shown in Scheme I.

Nitration[12] of *N*-(carboethoxy)-4-nitroanthranilic acid (**5**) using freshly distilled 100% nitric acid provided the 4,5-dinitro acid **6** in greater than 90% yield, while treatment of *N*-acetyl-4-nitroanthranilic acid under the same conditions provided the corresponding dinitro acid in 25–30% yield, as well as other regioisomers. Use of the less acid labile carboethoxy function as in **5** served not only to increase the selectivity and yield but also to provide C-2 of compound **8** later in the sequence.

Selective hydrolysis, well precedented in the quinazoline literature,[13] of the 8-ethylthio group of 6,8-bis(ethylthio)-*lin*-benzopurine (**9**), itself a versatile intermediate, provided 6-(ethylthio)-*lin*-benzohypoxanthine (**10**), the key intermediate for the preparation of 8-oxo-*lin*-benzopurine ribofuranosides. Ribosidation of **10** was accomplished by the mercuric salt method[14,15] to provide a 1:1 mixture of imidazole ribosides **11** and **12** in greater than 95% yield. *lin*-Benzoinosine (**3b**) was prepared from **12** and its regioisomer **13** from **11** by Raney nickel desulfurization and deacetylation with methanolic ammonia. The position of ribose attachment was confirmed by comparison with authentic samples of **3b** prepared by enzymatic deamination of *lin*-benzoadenosine (**1b**) with adenosine deaminase and by independent synthesis (vide infra). The substitution pattern of **1b** has been confirmed by ^{15}N–^{13}C and ^{15}N–^{1}H coupling in the NMR spectra of 1- and 3-substituted [^{15}N]-*lin*-benzoadenines[16] and by UV model studies.[1] Comparison of **3b** and **13** supports the trend that observed UV spectra of 1-substituted isomers of *lin*-benzopurines are red shifted with respect to the 3-substituted isomers.[1]

lin-Benzoguanosine (**2b**) was prepared by removal of the acetyl groups and displacement of the 6-ethylthio function of **12** with methanolic ammonia. This route to **2b** is

(1) Leonard, N. J.; Sprecker, M. A.; Morrice, A. G. *J. Am. Chem. Soc.* **1976**, *98*, 3987.

(2) The prefix *lin* refers to the linear disposition of the three rings in 1–4; "benzo" in the trivial name refers to the additional ring which, only when central, contains no nitrogen. This terminology is adaptable to derivatives similarly related to all purines. The other parts of the names followed accepted IUPAC–IUB nomenclature.

(3) Leonard, N. J.; Scopes, D. I. C.; VanDerlijn, P.; Barrio, J. R. *Biochemistry* **1978**, *17*, 3677.

(4) Scopes, D. I. C.; Barrio, J. R.; Leonard, N. J. *Science* **1977**, *195*, 296.

(5) VanDerLijn, P.; Barrio, J. R.; Leonard, N. J. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 4204.

(6) Schmidt, M. J.; Truex, L. T.; Leonard, N. J.; Scopes, D. I. C.; Barrio, J. R. *J. Cycl. Nucleotide Res.* **1978**, *4*, 201.

(7) Kauffman, R. F.; Lardy, H. A.; Barrio, J. R.; Barrio, M. d. C. G.; Leonard, N. J. *Biochemistry* **1978**, *17*, 3686.

(8) Thomas, R. W.; Leonard, N. J. *Heterocycles* **1976**, *5*, 839.

(9) Keyser, G. E.; Leonard, N. J. *J. Org. Chem.* **1976**, *41*, 3529.

(10) Leonard, N. J.; Morrice, A. G.; Sprecker, M. A. *J. Org. Chem.* **1975**, *40*, 356.

(11) Keyser, G. E.; Leonard, N. J., Paper presented in part at the 176th National Meeting of the American Chemical Society, Miami Beach, Fla., Sept 1978; American Chemical Society: Washington, D.C.; Abstr. ORGN 20.

(12) Goldstein, H.; Juanin, R. *Helv. Chim. Acta* **1951**, *34*, 1860.

(13) Armarego, W. L. F. "Fused Pyrimidines: Quinazolines"; Brown, D. J., Ed.; Wiley-Interscience: New York, NY, 1967; Vol. 24, pp 1, 283.

(14) Goodman, L. "Basic Principles of Nucleic Acid Chemistry"; Ts'o, P. O. P., Ed.; Academic Press: New York, NY 1974; Vol. 1, pp 94–194.

(15) Yamaoka, N.; Aso, K.; Matsuda, K. *J. Org. Chem.* **1965**, *30*, 149.

(16) Wiemer, D. F.; Scopes, D. I. C.; Leonard, N. J. *J. Org. Chem.* **1976**, *41*, 3051.

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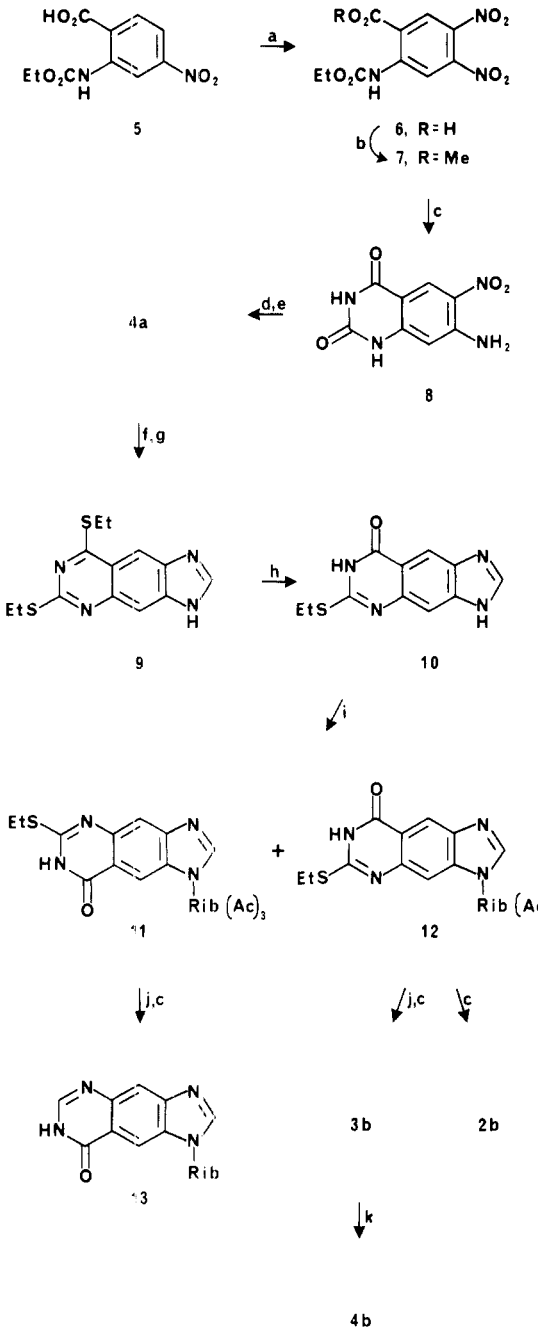
Scheme I[a]



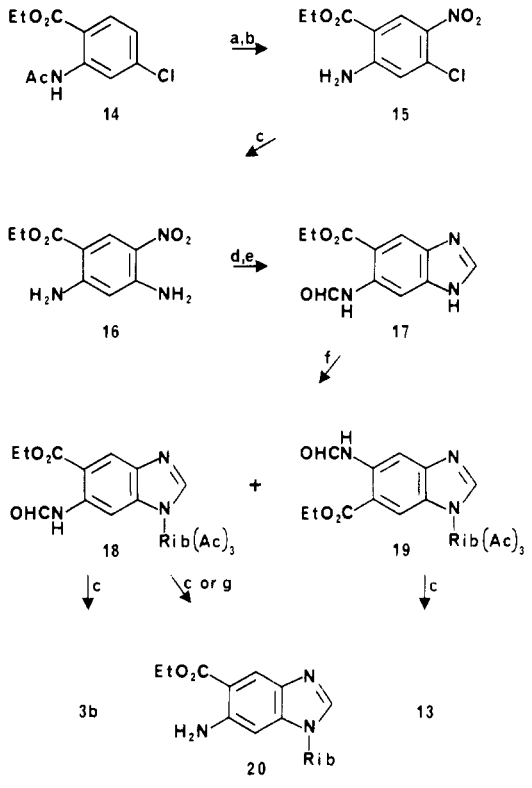
[a] The following conditions were used: a, 100% HNO_3; b, $(CH_3)_2SO_4$, $KHCO_3$; c, NH_3/CH_3OH; d, H_2, Pd/C, HCO_2H; e, HCO_2H, Δ; f, P_2S_5/pyridine; g, EtI, KOH; h, 20% KOH, *t*-BuOH, Δ; i, $(Ac)_3RibBr$, $Hg(CN)_2$; j, Raney Ni/EtOH; k, xanthine oxidase.

particularly useful in view of the low solubility and nonspecificity of reactivity in attempted direct ribosidations of *lin*-benzoguanine (**2a**).

lin-Benzoxanthosine (**4b**) was prepared from **3b** by enzymatic oxidation using xanthine oxidase. This reaction takes advantage of the unusual oxidation of a riboside by xanthine oxidase, the normal function of which is the oxidation of hypoxanthine to xanthine and subsequently to uric acid.[17] The laterally displaced ribose of this benzologue of inosine evidently allows access to and oxidation of C-6 (corresponding to C-2 in purines) while simultaneously blocking the normally facile[17] oxidation of

(17) Bray, R. C. *The Enzymes*, *2nd Ed.* **1963**, *3*, 533.

Scheme II[a]



[a] The following conditions were used: a, 90% HNO_3/H_2SO_4; b, EtOH, H_2SO_4; c, NH_3/CH_3OH; d, H_2, Pd/C, HCO_2H; e, HCO_2H, Δ, $PhCH_3$; f, $(Ac)_3RibBr$, $Hg(CN)_2$; g, EtONa/EtOH.

the imidazole ring by the enzyme. The notion of accessibility and blocking is supported by the fact that *lin*-benzo-IMP (**3c**) is oxidized to **4c** with nearly equal facility. It is ironic that enzymatic transformations could be used for obtaining **4b,c** from **3b,c**, since the latter compounds thereby do not behave analogously to the corresponding purine derivatives from which they were derived conceptually.

For direct preparations of *lin*-benzoinosine (**3b**), it was advantageous to use the route shown in Scheme II. The critical path determinant is the ring closure of **18** to **3b** (and **19** to **13**). The solution was found by a biomimetic approach to the problem, using the sensitive *N*-formyl protecting group as the source of C-6 in **3**. Less labile acyl protecting groups for the 6-amino function of 6-amino-5-(carboethoxy)benzimidazole led to analogues of formamido ribosides **18** and **19** without difficulty, except that an additional step was required for introduction of the groups. These analogues, inter alia, acetyl, chloroacetyl, and methoxyacetyl, did not give ring-closed products with methanolic ammonia, even under forcing conditions, nor were the groups capable of removal under conditions that left the newly formed glycosidic bond intact. In keeping with these results, attack by ammonia and primary amines may possibly occur first at the formyl carbon of **18** to give a fleeting tetrahedral intermediate, which can undergo either cleavage to give amino ester **20** or ring closure with the loss of water to give **3b**. No amino amide could be found either in the conversion of **18** to **3b** or in the reactions of the higher acyl analogues. The efficiency of ring closure vs. deprotection by ammonia is demonstrated by the 18:1 ratio of products **3b** and **20**. Similarly, compound **18** reacts with other unhindered primary amines to provide 7-alkyl-*lin*-benzoinosines, although the larger the amine, the more of **20** that is produced.[18] Preliminary results

indicate that treatment of 18 with excess sodium ethoxide in ethanol will remove both the *O*-acetyl and *N*-formyl protecting groups cleanly, if that is desired.

The tetrasubstituted benzenes ethyl 2-amino-4-chloro-5-nitrobenzoate and similar intermediates (Schemes I and II) provide great latitude in the preparation of the base portion of *lin*-benzopurines, allowing unequivocal substitution at nitrogens 3, 5, and 7 of the tricyclic systems. Treatment[19] of **15** with sodium hydride in dimethylformamide followed by addition of electrophiles produces substitution specifically at eventual N-5. The use of primary amines in place of ammonia in the conversion of **15** to **16** or **7** to **8** allows substitution at eventual N-3, and reaction of **17** with primary amines can provide N-7 substituted *lin*-benzohypoxanthines.

The utility of 6,8-bis(ethylthio)imidazo[4,5-*g*]quinazoline (**9**) is demonstrated by its conversion to 3-β-D-ribofuranosyl-6-(ethylthio)imidazo[4,6-*g*]quinazoline, 6-(ethylthio)-*lin*-benzoinosine (**12**), which leads to the benzologues of guanosine, inosine, and xanthosine, and by preliminary results indicating that ribosidation (of **9**) occurs smoothly to give two regioisomers and that treatment (of **9**) with methanolic ammonia followed by Raney nickel desulfurization provides *lin*-benzoadenine (**1a**).

The useful spectral properties[20] of most of the *lin*-benzopurines, their ribosides, and their ribotides and their enzymatic activity allow investigation of many biological interactions of purines. We have now provided a single, central pathway by which almost all the *lin*-benzopurine bases and ribosides can be obtained, plus an alternative method to allow for specific substitution. Work is continuing, utilizing these central intermediates to obtain fluorescent photoaffinity labels,[21] the higher phosphate derivatives, and benzologues of enzyme cofactors to investigate the scope and limitations of biological activity demonstrated by this system uniquely analogous to the purine series.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are corrected. Nuclear magnetic resonance spectra were recorded on a Varian EM-390 or HR-220 spectrophotometer, using tetramethylsilane (deuterated organic solvents) or acetone (deuterio aqueous solutions) as internal standards. Mass spectra were run on a Varian MAT CH-5 spectrometer (10 and 70 eV), coupled to a 620i computer and a STATOS recorder. Ultraviolet absorption spectra were obtained on a Beckman Acta M VI spectrophotometer. Microanalyses were performed by Mr. Josef Nemeth and his staff. Thin-layer chromatographs were run on EM silica gel f-254 plates (thickness 0.25 mm); Brinkmann 0.05–0.2 mm silica gel was used for column chromatography on silica. Bio-Rad Dowex 50W-X8 and Sigma TEAE and DEAE cellulose were used for ion-exchange chromatography.

All aqueous chromatographic columns were run at 5 °C. Triethylammonium bicarbonate, triethylammonium carbonate, and triethylammonium hydroxide are abbreviated TEAB, TEAC, and TEAH, respectively.

***N*-(Carboethoxy)-4-nitroanthranilic Acid (5).** A solution of 4-nitroanthranilic acid (30 g, 165 mmol) in hot, dry acetone (500 mL) was added in four portions at 3-h intervals to a mixture of ethyl chloroformate (75 mL) in dry acetone (250 mL) heated at reflux. The solution was heated at reflux for an additional 12 h, and the solvent was removed in vacuo. The yellow residue was crystallized from chloroform–ethanol as short white needles: 35.2 g (84%); mp 215 °C; MS *m/e* 254 (M^+); NMR ($(CD_3)_2SO$) δ 10.65 (br, 2, NH, OH), 9.00 (d, 1, 3-ArH, $J_{3,5}$ = 3 Hz), 8.08 (d, 1, 6-ArH, $J_{5,6}$ = 8 Hz), 7.76 (d of d, 1, 5-ArH, $J_{3,5}$ = 3 Hz, $J_{5,6}$ = 8 Hz), 4.20 (q, 2, OCH_2, J = 6 Hz), 1.33 (t, 3, CH_3, J = 6 Hz).

Anal. Calcd for $C_{10}H_{10}N_2O_6$: C, 47.25; H, 3.97; N, 11.02. Found: C, 47.18; H, 4.01; N, 10.91.

***N*-(Carboethoxy)-4,5-dinitroanthranilic Acid (6).** Finely powdered *N*-(carboethoxy)-4-nitroanthranilic acid (10 g, 39 mmol) was added in 10 portions at 5-min intervals to freshly distilled 100% nitric acid[22] (30 mL, d = 1.55 g/cm^3) at 0 °C under a nitrogen atmosphere. The solution was stirred at 0 °C for 4 h and at 25 °C for 2 h with formation of a white precipitate. The slurry was cooled to 0 °C and ice (30 g) was added slowly with vigorous stirring. The precipitate was filtered (funnel precooled to 0 °C) and washed successively with cold 30% HNO_3 (100 mL), cold 3% HNO_3 (100 mL), and cold water (100 mL). The material was dried in vacuo at 40 °C to yield 9.7 g (90%) of white powder, analytically pure; recrystallization, if necessary, could be accomplished from chloroform–ethanol: mp 217 °C; MS *m/e* 299 (M^+); NMR ($(CD_3)_2SO$) δ 11.1 (br, 2, OH, NH), 8.75 (s, 1, 6-ArH), 8.60 (s, 1, 3-ArH), 4.25 (q, 2, OCH_2, J = 6 Hz), 1.30 (t, 3, CH_3, J = 6 Hz).

Anal. Calcd for $C_{10}H_9N_3O_8$: C, 40.14; H, 3.03; N, 14.05. Found: C, 40.11; H, 3.07; N, 13.89.

Methyl *N*-(Carboethoxy)-4,5-dinitroanthranilate (7). To a solution of *N*-(carboethoxy)-4,5-dinitroanthranilic acid (5.0 g, 16.7 mmol) in acetone (500 mL) was added potassium bicarbonate[23] (5.0 g), water (5 mL), and dimethyl sulfate (5 mL). The solution was heated at reflux for 6 h and the solvent was removed in vacuo. The residue was dissolved in ethyl acetate (500 mL) and washed with 10% aqueous $KHCO_3$ (2 × 250 mL), the organic layer was dried (Na_2SO_4), and the solvent was removed in vacuo. The oily residue was crystallized from chloroform–ethanol to give 4.92 g (94%) of short, light yellow needles: mp 116 °C; MS *m/e* 313 (M^+); NMR ($(CD_3)_2SO$) δ 10.6 (br s, 1, NH), 8.70 (s, 1, 6-ArH), 8.60 (s, 1, 3-ArH), 4.23 (q, 2, OCH_2, J = 7 Hz), 3.93 (s, 3, OCH_3), 1.30 (t, 3, CH_3, J = 7 Hz).

Anal. Calcd for $C_{11}H_{11}N_3O_8$: C, 42.18; H, 3.54; N, 13.42. Found: C, 42.24; H, 3.69; N, 13.42.

7-Amino-6-nitroquinazoline-2,4-dione (8). Methyl *N*-(carboethoxy)-4,5-dinitroanthranilate (3.1 g, 10 mmol) was added to methanol (80 mL) saturated with ammonia at 0 °C in a sealed tube. The heterogeneous mixture was heated for 1 day at 60 °C and then 4 days at 110 °C. The solution was cooled to 0 °C, and the precipitate was filtered to give 2.3 g (96%) of yellow-orange crystals of the ammonium salt of **8**. The free acid (2.1 g, 95%) was obtained by heating the salt in ethanol (100 mL) at reflux for 2 h: mp >300 °C; MS *m/e* 222 (M^+); NMR ($(CD_3)_2SO$) δ 10.2 (br s, 1, NH), 10.1 (br s, 1, NH), 8.50 (s, 1, 5-ArH), 7.8 (br s, 2, NH_2), 6.50 (s, 1, 8-ArH).

Anal. Calcd for $C_8H_6N_4O_4$: C, 43.25; H, 2.72; N, 25.22. Found: C, 43.18; H, 2.94; N, 25.11.

(18) Treatment of 18 with methylamine in methanol gave 7-methyl-*lin*-benzoinosine (60%, by NMR) and **20** (40%, by NMR); cyclohexylamine gave **20** (ca. 60%) plus unidentified products.

(19) Keyser, G. E.; Gerzon, K.; Leonard, N. J. *J. Med. Chem.*, in preparation.

(20) A detailed study of the fluorescent properties of **2** and **4** will be presented elsewhere.

(21) Dreyfuss, G.; Schwartz, K.; Blout, E. R.; Barrio, J. R.; Liu, F.-T.; Leonard, N. J. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 1199.

(22) **Caution**! 100% HNO_3 is sensitive to heat and light; see: Stern, S. A.; Mallhaupt, J. T.; Kay, W. B. *Chem. Rev.* **1960**, *60*, 185.

(23) Use of potassium carbonate leads to formation of nitrophenolate impurities as well as alkylation of nitrogen.

***lin*-Benzoxanthine (4a).** Palladium on charcoal (10%, 0.2 g) was added to a heterogeneous mixture of 7-amino-6-nitroquinazoline-2,4-dione (8) (2.0 g, 9 mmol) in formic acid (98%, 200 mL) under argon and the solution was shaken under hydrogen (4 atm) for 2 h. The solution was filtered through a Celite pad, and the catalyst was washed with hot formic acid (98%, 2 × 100 mL). After the solution was heated at reflux for 2 h under nitrogen, the solvent was removed in vacuo. Formic acid (98%, 50 mL) and toluene (500 mL) were added to the residue, the heterogeneous solution was heated at reflux for 30 min, and the formic acid was removed by azeotropic distillation. Additional toluene (100 mL) was added, and the solvent was removed in vacuo. The solid residue was triturated with hot absolute ethanol and filtered to give 1.76 g (97%) of white powder: mp >300 °C; MS *m/e* 202; NMR (CF_3CO_2D) δ 9.47 (s, 1, 2-ArH), 8.90 (s, 1, 9-ArH), 7.93 (s, 1, 4-ArH).

The material was of sufficient purity for further use; an analytical sample could be obtained by dissolution in dilute sodium hydroxide and precipitation with acetic acid.

Anal. Calcd for $C_9H_6N_4O_2 \cdot {}^2/_3H_2O$: C, 50.47; H, 3.45; N, 26.16. Found: C, 50.76; H, 3.50; N, 26.19.

6,8-Bis(ethylthio)-*lin*-benzopurine (9). To a heterogeneous mixture of *lin*-benzoxanthine (**4a**) (1.6 g, 8 mmol) in dry pyridine (200 mL) was added purified phosphorus pentasulfide[24] (2.2 g, 10 mmol), and the solution was heated at reflux for 24 h. Additional phosphorus pentasulfide (2.2 g) was added in two portions at 24-h intervals, and heating was continued for an additional 16 h. The solution was concentrated to a volume of ca. 50 mL, poured into boiling water (1 L), and then boiled until hydrogen sulfide and pyridine were eliminated. The solution was treated with charcoal, filtered, and cooled to 0 °C. The yellow precipitate was filtered to give 6,8-dithio-*lin*-benzoxanthine (1.33 g, 71%).

The crude solid was added to 50% aqueous methanol (50 mL) and 1 M aqueous potassium hydroxide (5.8 mL). Ethyl iodide (1 mL, 12.5 mmol) was added to the yellow solution with vigorous stirring for 8 h. Acetic acid (1 mL) was added, and the methanol was removed in vacuo. The oily solution was extracted with ethyl acetate (5 × 50 mL), and the organic extracts were dried (Na_2SO_4), concentrated in vacuo, and applied to a column (2.5 × 40 cm) of silica gel (75 g) packed in chloroform (previously washed with 1 L of chloroform). Elution with a linear gradient of 0% (1 L) of 10% (1 L) ethanol in chloroform was followed by evaporation in vacuo of appropriate fractions of the eluent to give **9**: 1.57 g (68% from **4a**); mp 210 °C; MS *m/e* 290 (M^+); NMR ($(CD_3)_2SO$) δ 8.56 (s, 1, ArH), 8.20 (s, 1, ArH), 7.85 (s, 1, ArH), 6.04–4.5 (br, 1, NH), 3.33 (q, 2, SCH_2, *J* = 8 Hz), 3.23 (q, 2, SCH_2, *J* = 8 Hz), 1.40 (t, 6, $(SCH_2CH_3)_2$).

Anal. Calcd for $C_{13}H_{14}N_4S_2$: C, 53.76; H, 4.86; N, 19.29; S, 22.08. Found: C, 53.81; H, 4.92; N, 19.29; S, 21.73.

6-(Ethylthio)-*lin*-benzohypoxanthine (10). To a solution of 6,8-bis(ethylthio)-*lin*-benzopurine (**9**) (0.6 g, 2.1 mmol) and *tert*-butyl alcohol (50 mL) was added 20% aqueous potassium hydroxide (100 mL), and the solution was heated at reflux for 5 h. After neutralization with acetic acid the solvent was removed in vacuo. The solid yellow residue was triturated with water (25 mL), filtered, and washed with ice–water to give 486 mg of **10** (95%): mp 300 °C; MS *m/e* 246 (M^+); NMR ($(CD_3)_2SO$) δ 11.2 (s, 1, NH), 8.45 (s, 1, ArH), 8.25 (s, 1, ArH), 7.64 (s, 1, ArH), 7.0–5.0 (br, 1, NH), 3.22 (q, 2, *J* = 7 Hz), 1.36 (t, 3, *J* = 7 Hz).

Anal. Calcd for $C_{11}H_{10}N_4OS$: C, 53.64; H, 4.09. Found: C, 53.76; H, 4.05.

6-(Ethylthio)-1- and -3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-*lin*-benzohypoxanthine (11 and 12). To a solution of 6-(ethylthio)-*lin*-benzohypoxanthine (480 mg, 1.94 mmol) in hot, anhydrous DMF–nitromethane (1:10, 200 mL) was added mercuric cyanide (500 mg, 2 mmol). The heterogeneous solution was heated at reflux for 1 h and then allowed to cool to 40 °C under a positive pressure of nitrogen. 1-Bromo-tri-*O*-acetyl-β-D-ribofuranose (786 mg, 2.5 mmol)[1] in dry dichloromethane (50 mL) was added, and the solution was heated under nitrogen at 40 °C for 1 h and then at 100 °C for 18 h. The solvent was removed in vacuo, and the oily residue was dissolved in ethyl acetate (500 mL), washed with 30% aqueous potassium iodide (2 × 100 mL), dried (Na_2SO_4), and reduced to dryness in vacuo. The residue was dissolved in hot chloroform (10 mL) and applied to a column (4 × 80 cm) of silica gel (300 g) (previously washed with chloroform (4 L)) packed in chloroform. The products were eluted with a linear gradient of 0% (4 L) to 10% (4 L) ethanol in chloroform. Removal of solvent from the first major peak gave 460 mg (47%) of **11** as an off-white glass: NMR ($CDCl_3$) δ 11.0 (s, 1, NH), 8.45 (s, 1, ArH), 8.30 (s, 1, ArH), 8.00 (s, 1, ArH), 6.20 (d, 1, 1′-CH, *J* = 6 Hz), 5.5 (m, 3, 2′, 3′, and 4′-CH), 4.5 (m, 2, 5′-CH_2), 3.35 (q, 2, SCH_2, *J* = 8 Hz), 2.23 (s, 3, $C(O)CH_3$), 2.20 (s, 3, $C(O)CH_3$), 2.10 (s, 3, $C(O)CH_3$) 1.50 (t, 3, CH_3, *J* = 8 Hz).

Removal of solvent from the combined fractions corresponding to the second major peak gave 484 mg (49%) of **12** as a white powder: NMR ($CDCl_3$) 12.2 (s, 1, NH), 8.70 (s, 1, ArH), 8.20 (s, 1, ArH), 7.7 (s, 1, ArH), 6.11 (d, 1, 1′-CH, *J* = 6 Hz), 5.5 (m, 3, 2′, 3′, and 4′-CH), 4.4 (m, 2, 5′-CH_2), 3.30 (q, 2, SCH_2, *J* = 8 Hz), 2.27 (s, 3, $C(O)CH_3$), 2.17 (s, 3, $C(O)CH_3$), 2.07 (s, 3, $C(O)CH_3$), 1.47 (t, 3, CH_3, *J* = 8 Hz).

Both **11** and **12** were used without further purification.

***lin*-Benzoguanosine (2b).** To methanol saturated with ammonia at 0 °C (80 mL) in a sealed tube was added 6-(ethylthio)-2′,3′,5′-tri-*O*-acetyl-*lin*-benzoinosine (**12**) (100 mg, 2 mmol). After 24 h at room temperature, the tube was heated (oil bath) at 140 °C for 96 h. The tube was cooled to 0 °C and then opened, and the solution was reduced to dryness in vacuo. Purification was accomplished by either of two methods: the residue was dissolved in boiling water (*without* activated charcaol!) and filtered, and the volume of the filtrate was reduced by 50%. The gel that resulted after cooling was centrifuged, washed with 2-propanol, and dried in vacuo. For smaller quantities (1–10 mg), the material was dissolved in 0.05 M TEAC (pH 10), applied to a column of TEAE cellulose (3 × 40 cm), and eluted with a linear gradient from 0.05 M TEAC (1 L, pH 10) to saturated TEAC (1 L, pH 10). Appropriate fractions (UV) were concentrated in vacuo at 25 °C and coevaporated with methanol. The yield of **2b** was 61 mg (91%) as a white powder: UV $\lambda_{max}^{pH\ 8}$ (0.3 M TEAB) 335 (sh), 323 (ε 5600), 310 (sh), 288 (4100), 277 (5100) nm; MS *m/e* 333; NMR ($(CD_3)_2SO$, 5 mg/mL) δ 8.47 (s, 1, ArH), 8.16 (s, 1, ArH), 7.38 (s, 1, ArH), 6.5 (br, 3, NH and NH_2), 5.83 (d, 1, 1′-CH, *J* = 6 Hz), 4.37, 4.12, 3.98 (m's, 1 each, 2′, 3′, and 4′-CH), 3.66 (m, 2, 5′-CH), 3.32 (m's, 3, OH's).

(24) Purification was accomplished by extraction of technical grade (80%) phosphorus pentasulfide in a Soxhlet apparatus with carbon disulfide. Upon prolonged extraction, P_2S_5 in yellow needles of analytical purity is formed in the concentrate and can be isolated by filtration under argon. Use of technical grade reagent reduces yields of thiation to as little as 10%.

(25) Direct preparation of 0.05 M aqueous TEAB saturated the solution with carbon dioxide, limiting the enzymatic reaction proportionally to the concentration of oxygen present.

***lin*-Benzoinosine from 12.** Raney nickel (5 mg, No. 28, Grace Chemicals) was added to a solution of 6-(ethylthio)-2′,3′,5′-tri-*O*-acetyl-*lin*-benzoinosine (**12**) (5 mg, 0.01 mmol) in ethanol (5 mL). After the mixture had been heated at reflux for 2 h, the catalyst was removed by filtration and washed with hot ethanol (10 mL). The filtrate was saturated with ammonia at 0 °C and allowed to stand 24 h at room temperature in a sealed vessel. After removal of the solvent and trituration with ethyl acetate, the white solid obtained was found to be identical with an authentic sample[1] by UV and NMR spectroscopy and by the NMR spectrum of the mixture.

1-β-D-Ribofuranosyl-*lin*-benzohypoxanthine (13) from 11. Treatment of 6-(ethylthio)-2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl-*lin*-benzohypoxanthine as above gave **13**, identical with an authentic sample prepared according to Scheme II.

Ethyl *N*-Acetyl-4-chloroanthranilate (14). A solution of ethyl 4-chloroanthranilate[9] (7.0 g, 35 mmol) in acetic anhydride (11 mL) was heated at reflux for 3 h. After removal of solvent in vacuo, the solid residue was recrystallized from light petroleum ether to give 8.3 g (98%) of white needles: mp 78 °C; MS *m/e* 241/243 (M^+); NMR ($CDCl_3$) δ 11.0 (br, 1, NH), 8.7 (d, 1, 3-ArH), 7.9 (d, 1, 6-ArH), 6.9 (d of d, 1, 5-ArH), 4.3 (q, 2, CH_2), 2.2 (s, 3, $COCH_3$), 1.4 (t, 3, CH_3).

Anal. Calcd for $C_{11}H_{12}ClNO_3$: C, 54.66; H, 5.01; N, 5.80. Found: C, 54.59; H, 5.03; N, 5.97.

Ethyl 2-Amino-4-chloro-5-nitrobenzoate (15). Ethyl *N*-acetyl-4-chloroanthranilate (**14**) (5.0 g, 20.4 mmol) was added in portions to a solution of fuming nitric acid (90%, 1.25 mL) in concentrated sulfuric acid (98%, 10 mL) at −20 °C with vigorous stirring. When dissolution was complete, the solution was warmed to 45 °C for 1 h and then was poured onto ice (100 g). The yellow solid isolated by filtration was dissolved in ethanol (200 mL), sulfuric acid (98%, 2 mL) was added, and the solution was heated at reflux for 30 min. After removal of the solvent in vacuo, the oily residue was dissolved in ethyl acetate (200 mL) and washed with 10% aqueous sodium bicarbonate (2 × 100 mL). Evaporation of the solvent in vacuo gave a yellow solid which was recrystallized successively from ethanol and benzene to give 5.5 g of yellow needles (93%): mp 163.5 °C; NMR ($CDCl_3$) δ 8.6 (s, 1, 6-ArH), 6.7 (s, 1, 3-ArH), 6.0 (br, 2, NH_2), 4.4 (q, 2, CH_2), 1.4 (t, 3, CH_3).

Anal. Calcd. for $C_9H_9ClN_2O_4$: C, 44.18; H, 3.71; N, 11.45. Found: C, 44.33; H, 3.75; N, 11.64.

Ethyl 2,4-Diamino-5-nitrobenzoate (16). To ethanol saturated with ammonia at 0 °C (80 mL) was added ethyl 2-amino-4-chloro-5-nitrobenzoate (**15**) (4.0 g, 16 mmol). The tube was heated (oil bath) at 130 °C for 36 h, cooled to 0 °C, and then opened. The solvent was removed in vacuo, and the residue was triturated with cold ethanol (2 × 50 mL) to give 3.5 g (94%) of **16**: MS *m/e* 225 (M^+); NMR ($(CD_3)_2SO$) δ 8.60 (s, 1, 6-ArH), 7.45 (s, 2, NH_2), 7.25 (s, 2, NH_2), 6.00 (s, 1, 3-ArH), 4.23 (q, 2, OCH_2, J = 7 Hz), 1.33 (t, 3, CH_3, J = 7 Hz).

The material was used without further purification.

5-(Carboethoxy)-6-formamidobenzimidazole (17). Ethyl 2,4-diamino-5-nitrobenzoate (**16**) (2.25 g, 10 mmol) and 10% palladium on charcoal (1.1 g) were added to absolute ethanol (150 mL) and stirred vigorously under a positive pressure of nitrogen. Hydrazine hydrate (99%, 1.5 mL) in absolute ethanol (25 mL) was added dropwise, and the solution was stirred for 3 h. The solution was reduced to dryness in vacuo at 25 °C in the presence of catalyst, then benzene (200 mL) was added, and the whole was evaporated in vacuo. Formic acid (98%, 50 mL) was added, and the solution was heated at reflux for 3 h under nitrogen. The volume of the solution was reduced to ca. 25 mL in vacuo and toluene (200 mL) was added. The remaining formic acid was removed by azeotropic distillation at atmospheric pressure until 100 mL of toluene was distilled at 110 °C, maintaining the volume of the solution by addition of toluene at regular intervals. The hot solution was filtered through Celite and refrigerated overnight. The white powder (1.95 g) thus obtained was shown to contain less than 5% 6-amino-5-(carboethoxy)benzimidazole by NMR and was sufficiently pure to use in subsequent reactions: mp 186 °C; MS *m/e* 233 (M^+); NMR ($(CD_3)_2SO$) δ 11.0 (br, 1, NH), 9.5–7.5 (br, 1, NH), 8.9 (s, 1, 2-ArH), 8.8 (s, 1, NCHO), 8.6 (s, 1, ArH), 8.5 (s, 1, ArH), 4.5 (q, 2, CH_2), 1.4 (t, 3, CH_3).

***lin*-Benzoinosine (3b) and 1-(β-D-Ribofuranosyl)-*lin*-benzohypoxanthine (13).** Mercuric cyanide (1.70 g, 6.7 mmol) was added to a solution of 5-(carboethoxy)-6-formamidobenzimidazole (**17**) (1.5 g, 6.7 mmol) in nitromethane (300 mL) and heated at reflux for 1 h. A portion (50 mL) of the solvent was removed by distillation to ensure dryness, and the yellow solution was cooled to ca. 40 °C under a positive pressure of dried ($CaSO_4$) nitrogen. A solution of freshly prepared 1-bromo-2,3,5-tri-*O*-acetylribofuranose (from tetra-*O*-acetyl-β-D-ribofuranose (2.6 g, 8.2 mmol)[1] in anhydrous nitromethane (50 mL) was added, and stirring was continued for 1 h at 50 °C. The heterogeneous solution was then heated at reflux for 12 h and reduced in vacuo to give a clear brown oil. The oil was dissolved in chloroform (300 mL) and washed successively with 30% aqueous potassium iodide (3 × 200 mL) and water (3 × 200 mL). The chloroform solution was dried ($MgSO_4$), reduced in vacuo to a volume of 10 mL, and applied to a column of silica gel (500 g, 5 × 70 cm, washed with 4 L of chloroform). The column was eluted with chloroform (2 L) and then with a linear gradient from 0% (5 L) to 5% (5 L) ethanol in chloroform, collecting 25-mL fractions. The appropriate fractions were combined and reduced in vacuo to give **18** (1.61 g, 49%) and **19** (1.54 g, 46%): NMR of **18** ($CDCl_3$) δ 11.35 (br, 1, NH), 8.91 (s, 1, NCHO), 8.52 (s, 2, ArH), 8.23 (s, 1, ArH), 6.13 (d, 1, 1′-CH, J = 6 Hz), 5.5 (m, 3, ribH), 4.43 (m, 2, 5′-CH_2), 4.39 (q, 2, OCH_2, J = 7 Hz), 2.20 (s, 3, CH_3), 2.13 (s, 3, CH_3), 2.10 (s, 3, CH_3), 1.43 (t, 3, CH_3, J = 7 Hz). Further elution with 5% ethanol in chloroform gave slightly impure unribosidated material (**17**) (0.05 g, 3%). The first major compound eluted, **18**, was dissolved at 0 °C in methanol (180 mL) which had been saturated with ammonia at 0 °C. After standing at room temperature in a sealed tube for 96 h, the solution was reduced to dryness in vacuo. The white powder remaining was dissolved in 0.03 M aqueous TEAH (5 mL) and applied to a TEAE cellulose column (3 × 40 cm), and this was eluted with 0.03 M aqueous TEAH (250 mL) and then with a pH gradient from 0.3 M aqueous TEAH (1 L) to 0.1 M aqueous TEAB (1 L, pH 7.6). Compound **20** was eluted with the solvent front, slightly impure (TLC); evaporation of fractions containing this fluorescent material gave 51 mg of tan solid. Compound **3b** was eluted when the pH of the effluent dropped below 10; the appropriate fractions (by A_{326}) were reduced in vacuo to dryness and coevaporated with methanol (3 × 100 mL) to decompose any remaining buffer to give 902 mg (86%) of white solid, **3b**, identical by UV, TLC, and NMR–mixed NMR to the product obtained by enzymatic deaminolysis of *lin*-benzoadenosine: MS *m/e* 318 (M^+); UV $\lambda_{max}^{pH\,7}$ (0.1 M phosphate) 326 (ε 6300), 313 (6300), 298 (5600) nm; NMR (0.001 M TEAH in D_2O) δ 8.2 (s, 1, ArH), 7.6 (s, 1, ArH), 7.5 (s, 1, ArH), 7.1 (s, 1, ArH), 5.7 (d, 1,

1′-H), 4.4 (t, 1, ribH), 4.2 (t, 1, ribH), 4.1 (g, 1, ribH), 3.8 (m, 2, 5′-CH_2).

Anal. Calcd for $C_{14}H_{14}N_4O_5$: C, 52.83; H, 4.43. Found: C, 52.92; H, 4.55.

Similar treatment of **19** gave **13**: UV $\lambda_{max}^{pH\ 7}$ (0.1 M phosphate) 336, 322, 310, 283, 264 nm.

***lin*-Benzoxanthosine (4b).** To a solution of 0.05 M aqueous TEAB (pH 7.6, 970 mL, made by diluting 0.5 M aqueous TEAB)[25] was added a solution of *lin*-benzoinosine (**3b**) (100 mg, 0.31 mmol) in 0.05 M aqueous TEAH (30 mL). A solution of xanthine oxidase (20 units in 1.5 mL of 2.3 M aqueous ammonium sulfate, Sigma Grade I) was added, and the solution was agitated for 1 min. After 30 min, there was no further decrease in A_{298} and the solution was diluted with an equal volume of methanol and immersed in a dry ice–acetone bath for 30 min. Removal of the solvent in vacuo at 0–5 °C gave a yellow glass which solidified on successive coevaporations with methanol (250 mL) and ethanol (250 mL). The residue was dissolved in 0.1 M aqueous TEAH (10 mL), applied to a TEAE cellulose column (3 × 40 cm), and eluted with a pH gradient from 0.04 M (1 L) aqueous TEAH to 0.08 M (1 L) aqueous TEAB (pH 7.6). Appropriate fractions (by A_{320}) were concentrated in vacuo at 25 °C and dried by evaporation with pyridine (3 × 50 mL) to yield 102 mg (98%) of **4b**: UV $\lambda_{max}^{pH\ 7.6}$ (0.05 M TEAB) 321 (ϵ 6300), 285 (4700), 270 (6900) nm; field desorption MS m/e 334 (M^+).

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Registry No. 2b, 70631-11-7; **3b**, 60189-63-1; **4a**, 60189-64-2; **4b**, 60189-65-3; **5**, 70631-12-8; **6**, 70631-13-9; **7**, 70631-14-0; **8**, 70631-15-1; **8** ammonium salt, 70631-16-2; **9**, 70631-17-3; **10**, 70631-18-4; **11**, 70631-19-5; **12**, 70631-20-8; **13**, 70631-21-9; **14**, 63243-77-6; **15**, 70631-22-0; **16**, 70631-23-1; **17**, 70631-24-2; **18**, 70631-25-3; **19**, 70631-26-4; **20**, 70631-27-5; 4-nitroanthranilic acid, 619-17-0; ethyl chloroformate, 541-41-3; 6,8-dithio-*lin*-benzoanthine, 70631-28-6; 1-bromotri-*O*-acetyl-β-D-ribofuranose, 39925-22-9; ethyl 4-chloroanthranilate, 60064-34-8; 7-methyl-*lin*-benzoinosine, 70631-29-7.

Reduction–Elimination of Some Vicinal Cycloalkyl Cyanohydrin Derivatives. Stereoselective Synthesis of Cycloalkenes

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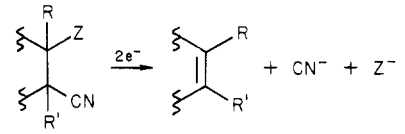
The isomeric 2-cyano-2-methylcyclododecanols, 2-cyano-1,2-dimethylcyclododecanols, and 2-cyano-1,2-dimethylcyclohexanols were prepared from the corresponding cyano ketones. Reduction of the methanesulfonate, (methylthio)methyl, and (methanesulfonyl)methyl derivatives with lithium in ammonia (Li/NH_3) or sodium naphthalenide ($NaC_{10}H_8$) gave rise to *cis*- and/or *trans*-1-methylcyclododecene, 1,2-dimethylcyclododecene, and 1,2-dimethylcyclohexene, respectively. The cyclododecyl systems showed a high preference for syn elimination with $NaC_{10}H_8$ whereas Li/NH_3 gave products of both syn and anti elimination. The findings suggest a preferred coplanar transition state for the elimination reactions.

We have found the reductive elimination of vicinal cyanohydrin derivatives to be a useful method for the synthesis of tri- and tetrasubstituted cycloalkenes (Scheme I).[1,2] We recently noted that sodium naphthalenide in hexamethylphosphoramide ($NaC_{10}H_8$/HMPA) effected a highly stereoselective syn elimination of certain cyclododecyl cyanohydrin derivatives in high yield.[2] We now report additional studies along these lines which show that the elimination reaction can also take place via an anti pathway under some circumstances.

The cyanohydrin derivatives chosen for these studies were prepared as follows (Chart I). Reduction of 2-cyano-2-methylcyclododecanone (**1a**) with sodium borohydride in isopropyl alcohol afforded the crystalline trans and cis cyanohydrins **2a** and **3a** as a 55:45 mixture in nearly quantitative yield. This mixture, and the derived methanesulfonates **4a** and **5a**, could be separated conveniently by high-pressure liquid chromatography (LC).

Scheme I



The (methylthio)methyl (MTM) ethers **6a** and **7a** were formed nearly quantitatively upon treatment of the cyanohydrins **2a** and **3a** with dimethyl sulfoxide in acetic anhydride–acetic acid.[3] These could also be separated by LC. Oxidation with *m*-chloroperoxybenzoic acid gave the crystalline sulfones **8a** and **9a**.

The ditertiary cyanohydrins **10a** and **11a** were produced as a 75:25 mixture in 88% yield upon addition of methylmagnesium bromide to ketone **1a**. Earlier we had found that cyano ethers such as I (R = R′ = CH_3, Z = OCH_3) underwent reductive elimination to olefins with dissolving metals.[1] Accordingly we prepared ethers **12a**

(1) J. A. Marshall and L. J. Karas, *Synth. Commun.*, 8, 65 (1978).
(2) J. A. Marshall and L. J. Karas, *J. Am. Chem. Soc.*, **100**, 3615 (1978).
(3) P. M. Pojer and S. Angyal, *Tetrahedron Lett.*, 3067 (1976).

0022-3263/79/1944-2994$01.00/0