M solution in hexane, 0.1 mL). After 5 min, CH_3OD (0.1 mL) was added and the mixture was allowed to warm to room temperature. It was evaporated onto a small amount of silica gel and chromatographed on a 10-g silica gel column with 10% ether–petroleum ether as the eluting solvent. The major product (22 mg, 68%) was identified by its UV spectrum[17] as 3-methoxyisoquinoline; its NMR spectrum lacked a singlet at ca. 9 ppm, indicating that the 1-position was deuterated. A minor fraction with dark blue fluorescence was also recovered. This was tentatively identified as a mixture containing 1-butyl-3-methoxyisoquinoline and 3-methoxy-1-methylisoquinoline on the basis of mass spectrometry, including exact mass measurements on the peaks at m/e 215 and 173, and NMR, which showed no signals near 9 ppm, indicative of 1-substitution of the isoquinolines. We suspect that the 1-methyl compound, which gave the most intense peak in the mass spectrum, arose via methylation of the anion by starting material; for this reason, we tried to prepare the anion in dilute solution and to trap it promptly with an excess of the desired reagent. On another occasion, 3-methoxyisoquinoline was isolated from a failed reaction; it was identified by its UV spectrum and by ^1H NMR: (200 MHz, $CDCl_3$) δ 4.03 (s, 3, OCH_3), 7.00 (s, 1, 4-H), 7.33–7.40 (ddd, 1), 7.52–7.60 (ddd, 1), 7.68 (d, J = 8 Hz, 1, 5-H), 7.88 (d, J = 8 Hz, 1, 8-H), 8.95 (s, 1, 1-H).

3-Methoxyisoquinoline-1-carboxaldehyde (27). A solution of **26** (250 mg, 1.05 mmol) in THF (100 mL) was cooled under N_2 to −78 °C and 1.1 equiv of n-butyllithium was added. The solution immediately became yellow. DMF (1.62 mL, 21 mmol, distilled under reduced pressure from CaH_2 and stored over CaH_2) was added via syringe within 5 s; the solution at once became lighter in color. After warming to room temperature, the reaction mixture was added to saturated NH_4Cl solution (120 mL) and the phases were separated. The aqueous layer was extracted with CH_2Cl_2 (2 × 50 mL); the combined organic layers were dried ($MgSO_4$) and evaporated to an oil which was chromatographed on a 30-g silica gel column in 50% $CHCl_3$–petroleum ether. The central tube among the fractions containing product was evaporated separately from the rest, which contained traces of fluorescent impurities. The central fraction was dried overnight at 38 °C under high vacuum to afford an analytically pure yellow solid (16%); on TLC, the product had a pale blue fluorescence. The slightly impure material (71% overall yield) was adequate for further transformation: mp 86–87 °C; ^1H NMR (200 MHz, $CDCl_3$) δ 4.07 (s, 3, OCH_3), 7.17 (s, 1, 4-H), 7.40–7.60 (m, 2), 7.66 (d, J = 8 Hz, 1, 5-H), 9.11 (d, J = 8 Hz, 1, 8-H), 10.25 (s, 1, CHO); IR (mineral oil) 1700 (s), 1590 (s), 1310 (s), 1240, 1185, 1052, 885, 875 cm^{-1}; UV (EtOH) λ_{max} (log ϵ) 380 (3.36), 346 (3.57), 289 (3.20), 277 (3.38), 257 nm (sh, 3.75); MS (10 eV), m/e (relative intensity) 187 (100, M^+), 186 (53), 158 (33), 143 (18), 130 (30), 116 (31), 102 (26), 89 (25); HREIMS found m/e 187.0632, $C_{11}H_9NO_2$ 187.0633. Anal. Calcd for $C_{11}H_9NO_2$: C, 70.57; H, 4.86; N, 7.48. Found: C, 70.53; H, 4.83; N, 7.44.

Wittig Reaction of 27. On a 20- to 50-mg scale, the aldehyde **27** was caused to react with $(Ph_3PCH_2OCH_3)^+Cl^-$ using either EtO^- or phenyllithium as the base, as described by Wittig and Schlosser[18,24] for reaction of this salt with benzaldehyde. A fluorescent product with the same R_f (TLC) as the product derived from 1-methoxy-2-azabiphenylene was isolated in 9–15% yield by silica gel chromatography in 50% $CHCl_3$–petroleum ether. The identity of this product with compound **25** was established by ^1H NMR, UV, and IR spectroscopy.[25]

Acknowledgment. This work was supported by Research Grant GM-05829 from the National Institutes of Health. High-resolution mass spectral data were obtained in part under a grant from the National Institute of General Medical Sciences (GM-27029). NMR data were obtained in part with support from the University of Illinois NSF Regional Instrumentation Facility, Grant NSF CHE 79-16100.

Registry No. **1**, 87954-15-2; **7**, 87954-17-4; **8**, 96246-03-6; **9**, 96246-06-9; **10**, 2402-91-7; **11**, 96245-98-6; **12**, 96246-01-4; **13**, 96246-02-5; **14**, 96245-99-7; **15**, 96246-04-7; **16**, 96246-05-8; **17**, 96246-00-3; **18**, 18677-43-5; **18**·picrate, 93087-25-3; **19**, 620-08-6; **20**, 96246-07-0; **25**, 96246-08-1; **26**, 55086-52-7; **27**, 96246-10-5; TMSC≡CH, 1066-54-2; TMSC≡CTMS, 14630-40-1; o-$O_2NC_6H_4CH_2CO_2H$, 3740-52-1; $(Ph_3PCH_2OCH_3)^+Cl^-$, 4009-98-7; 2,4-dihydroxypyridine, 84719-31-3; 3-bromo-2,4-dihydroxypyridine, 96245-97-5; 3-methoxyisoquinoline, 16535-84-5; 1-butyl-3-methoxyisoquinoline, 96246-09-2; 3-methoxy-1-methylisoquinoline, 23832-77-1.

(24) A valuable modification of this reaction has been reported: Earnshaw, C; Wallis, C. J.; Warren, S. *J. Chem. Soc., Perkin Trans. 1* **1979**, 3099.

(25) The Z isomer was not isolated, although its presence was suggested by a pair of doublets (δ 5.96 and 6.48 ppm, J = 7 Hz) in the ^1H NMR (200 MHz, $CDCl_3$) spectrum of an impure, more polar fraction of the reaction mixture. These chemical shift and J values correspond well to those reported for several (2-methoxyethenyl)naphthalenes.[16] The isolation of the Z isomer by preparative TLC (silica, 5% EtOH–$CHCl_3$) was complicated by its slow ($t_{1/2}$ > 2 h) isomerization to the E isomer on silica gel in the solvent used. The slowness of this isomerization reassures us that the E configuration of the product from **9** did not result from an artifact of chromatography.

Annelation of Guanosine by Reaction with Methyl *N*-Cyanomethanimidate and Sodium Methoxide To Give a Tricyclic, Fluorescent Analogue of Adenosine

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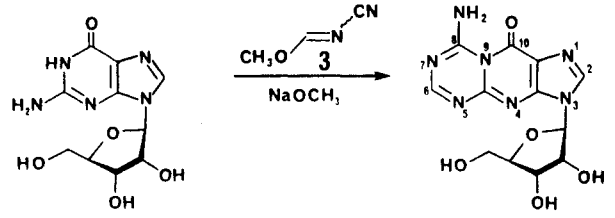
A reagent consisting of methyl *N*-cyanomethanimidate and sodium methoxide in methanol converts guanosine to a fluorescent product, 8-amino-3,10-dihydro-10-oxo-3-β-D-ribofuranosyl-1,3,5-triazino[1,2-*a*]purine. The tricyclic *N*-ribonucleoside thus formed resembles adenosine in its periphery and is an inhibitor of adenosine deaminase. This annelation of guanosine is the first example of a potentially general transformation of a natural *N*-ribonucleoside into an entity whose structure more closely resembles, in the periphery, that of a different natural *N*-ribonucleoside. The product can also serve as a "protected" guanosine since it reverts readily to guanosine upon treatment with dilute aqueous alkali. The reagent itself can be used in parallel with chloroacetaldehyde as a spray for fluorescence detection of guanosine and adenosine and differentiation between these on chromatograms. Guanosine, 8-bromoguanosine, 9-benzylguanine, and 9-benzyl-3-bromoguanine were used as representative substrates for the annelation reaction.

The purpose of this enterprise was to find the means of converting a natural *N*-ribonucleoside into a structure that more closely resembles, in the periphery, that of a different natural *N*-ribonucleoside.[1] We have chosen to attach the

0022-3263/85/1950-2468$01.50/0

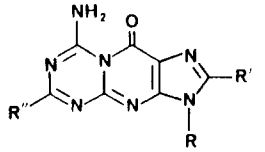
trivial (perhaps artificial) name "metamorphosine" to such a product to indicate a change in form. The conversion of guanosine (1), as an example, into a product that bears a peripheral resemblance to adenosine, such as 2, requires the addition of a CNCN moiety at the correct level of hydrogen content. Best results have been obtained with methyl *N*-cyanomethanimidate (3)[2-6] in the presence of sodium methoxide under strictly anhydrous conditions. The structure of the fluorescent product from 1 and 3 was established by spectroscopic means, including X-ray crystallographic analysis of an analogue, as 8-amino-3,10-dihydro-10-oxo-3-β-D-ribofuranosyl-1,3,5-triazino[1,2-*a*]-purine (2). We use the trivial name "IA′-metamorphosine" for 2 to indicate in a formal sense the metamorphosis of an inosine (disconnection of the terminal ring) into an adenosine-like molecule.[7]



1 2

Whereas compound 2 incorporates a new aminotriazine onto the existing natural ribosylpurine 1, it still possesses an imidazole ring, ribosyl moiety, and the major hydrogen-bonding functionalities generally considered important for the expression of the biological activity of the purine ribonucleosides and ribonucleotides.[8,9] The IA′-metamorphosine (2) may also be regarded as a laterally extended adenosine analogue in which the terminal rings are displaced in the range of 2.4 Å relative to adenosine and, as such, bears a resemblance to *lin*-benzoadenosine,[10,11] with related potential for application in enzyme studies. The conversion of N-bicyclic to N-tricyclic ribonucleosides by means of simple reagents has already provided interesting results and useful applications.[12-18]

Table I. Selected ^{1}H NMR Data for IA′-Metamorphosine and Related Compounds



R	R′	R″	triazine CH	imidazole CH	NH_2
Rib	H	H	8.03	8.30	9.39, 10.20
Rib	D	H	8.09		9.43, 10.26
Rib	H	D		8.34	9.39, 10.22
Rib	Br	H	8.06		9.40, 10.15
Bn	H	H	8.03	8.23	9.37, 10.25
Bn	Br	H	8.07		9.46, 10.18

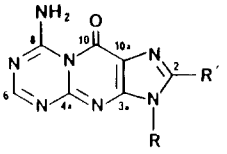
Methyl *N*-cyanomethanimidate (3) is a reagent of choice for annelation of a CNCN unit onto α-amino heterocycles.[2-6] However, the use of reagent 3 is restricted due to side reactions resulting from further reaction with cyanamide, liberated from 3 at elevated temperatures.[2] Hence, it was necessary to use mild conditions for the intended conversion. Since it was later found that the product (2) was unstable to aqueous alkali, it was also necessary to maintain anhydrous conditions during its preparation. Methyl *N*-cyanomethanimidate (3) (7 equiv)[2-6] was introduced through a hypodermic syringe into guanosine (1) and sodium methoxide (2 equiv) prepared from sodium in anhydrous methanol, and the mixture was stirred at 20 °C for 24 h. Purification was effected by partial evaporation, filtration, and pressure chromatography on Woelm silica gel with acetone as eluant. Evaporation gave a colorless solid, mp 247–249 °C dec, $C_{12}H_{13}N_7O_5$, in 39% yield (66% based on unrecovered guanosine). The same product could be obtained from guanosine, sodium hydride, and 3 in DMF at 60 °C for 6 h in about the same yield.

The ^{1}H NMR spectrum of the $C_{12}H_{13}N_7O_5$ product in $(CD_3)_2SO$ indicated two distinct exocyclic N–H signals that were exchangeable in D_2O. The fact that there were clearly differentiated chemical shifts at δ 10.2 and 9.39 suggested hydrogen bonding between the *peri*-carbonyl and the proximate exocyclic N–H. Since 2 represents the only structure among the four isomeric possibilities arising from different modes of condensation–cyclization of 1 (N^2-1 vs. N^2-3) and 3 (C=N vs. C≡N) that contains such readily differentiated exocyclic N–H's, we proceeded on the assumption that structure 2 was correct. At the same time, we carried out experiments that would eventually provide unequivocal confirmation of this initial assumption (see below).

Assignments of the ^{1}H and ^{13}C NMR chemical shifts for the nuclei that are part of the N-heterocyclic system were obtained through selective deuteriation of the triazine C–H and imidazole C–H in 2. Preparation of [8-^{2}H]guanosine (4) with greater than 93% deuterium incorporation was accomplished by heating guanosine (1) in D_2O under reflux for 7 h, following a similar procedure for the 8-deuteriation of adenosine.[19] The reaction of compound 4 with methyl *N*-cyanomethanimidate (3) yielded IA′-metamorphosine bearing deuterium in the imidazole ring (5). In the spectrum of 5, the singlet at δ 8.3 had less than 10% of the intensity of the singlet at δ 8.03 and was therefore assigned

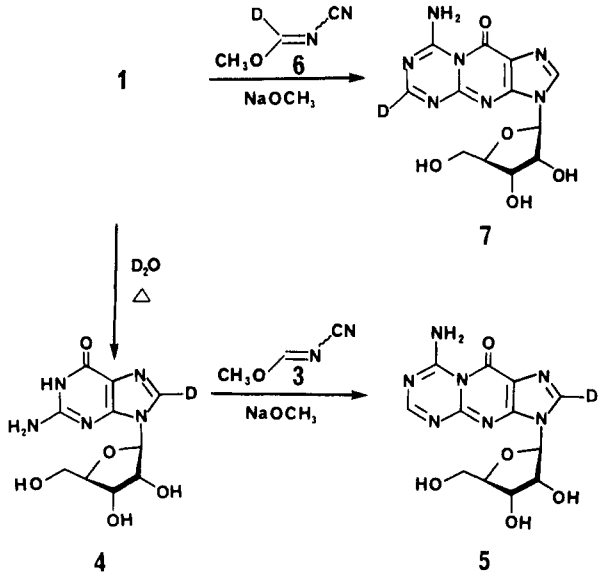
(1) Leonard, N. J.; Hosmane, R. S.; Agasimundin, Y. S.; Kostuba, L. J.; Oakes, F. T. *J. Am. Chem. Soc.* **1984**, *106*, 6847.
(2) Hosmane, R. S.; Leonard, N. J. *J. Org. Chem.* **1981**, *46*, 1457 and references therein.
(3) Hosmane, R. S.; Bakthavachalam, V.; Leonard, N. J. *J. Am. Chem. Soc.* **1982**, *104*, 235.
(4) Hosmane, R. S.; Rossman, M. A.; Leonard, N. J. *J. Am. Chem. Soc.* **1982**, *104*, 5497.
(5) Shahbaz, M.; Urano, S.; LeBreton, P. R.; Rossman, M. A.; Hosmane, R. S.; Leonard, N. J. *J. Am. Chem. Soc.* **1984**, *106*, 2805. Interchange numbers of Schemes II and III, formulas 5 and 6.
(6) Oakes, F. T.; Leonard, N. J. *J. Org. Chem.*, submitted for publication.
(7) An alternative trivial name, "GA′-metamorphosine", that might be used to designate the starting material and the peripheral appearance of the product, would be cumbersome in general application because, with other reagents, different starting materials could lead to the same product. The authors recognize that the proposed name may not be popular, but we find it useful.
(8) Olsson, R. A. *Biochemistry* **1978**, *17*, 367.
(9) Rosinová, M.; Holý, A.; Zelinková, E. *Collect. Czech. Chem. Commun.* **1978**, *43*, 2330.
(10) Leonard, N. J. *Acc. Chem. Res.* **1982**, *15*, 128.
(11) Leonard, N. J. "Biologically Active Principles of Natural Products"; Voelter, W., Daves, D. G., Eds.; Georg Thieme Verlag: Stuttgart, 1984; pp 237–249.
(12) Chung, F.-L.; Earl, R. A.; Townsend, L. B. *Tetrahedron Lett.* **1980**, *21*, 1599.
(13) Chung, F.-L.; Earl, R. A.; Townsend, L. B. *J. Org. Chem.* **1980**, *45*, 2532.
(14) Bhat, G. A.; Townsend, L. B. *J. Chem. Soc., Perkin Trans. 1* **1981**, 2387.
(15) Chung, F.-L.; Schram, K. H.; Panzica, R. P.; Earl, R. A.; Wotring, L. L.; Townsend, L. B. *J. Med. Chem.* **1980**, *23*, 1158.
(16) Townsend, L. B.; Bhat, G. A.; Chung, F.-L.; Schram, K. H.; Panzica, R. P.; Wotring, L. L. *INSERM Symp. Ser. (Paris)* **1979**, *81*, 37.
(17) Leonard, N. J. *CRC Crit. Rev. Biochem.* **1984**, *15*, 125 and references therein [Note: interchange pages 157 and 158].
(18) Nair, V.; Turner, G. A.; Offerman, R. J. *J. Am. Chem. Soc.* **1984**, *106*, 3370.
(19) Fox, J. R. Ph.D. Thesis, University of Illinois, 1965.

Table II. Selected ^{13}C NMR Data for IA′-Metamorphosine and Related Compounds



	R = Rib; R′ = H	R = Bn; R′ = H	R = Bn; R′ = Br	R = Rib; R′ = Br
C2	140.00	141.83	127.76	127.06
C3a	148.96	149.03	151.88	150.32
C4a/8	150.97	150.93	152.83	151.56
C6	161.57	161.42	163.17	162.58
C8/C4a	158.51	158.65	159.88	158.83
C10	157.55	157.73	157.90	156.87
C10a	119.10	118.73	120.35	120.34

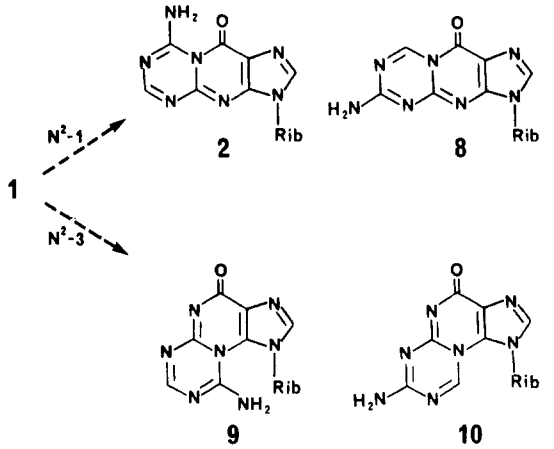
to the imidazole hydrogen. For deuterium labeling in the triazine ring, methyl [1-^{2}H]-*N*-cyanomethanimidate (**6**), prepared by the reaction of trimethyl [1-^{2}H]orthoformate with cyanamide in cyclohexane at reflux with the continuous azeotropic removal of methanol by using a Dean–Stark trap, was caused to react with guanosine to give compound **7**. The ^{1}H NMR spectrum of **7** showed >90% diminution in the intensity of the singlet at δ 8.03 present in the spectrum of **2** (Table I).



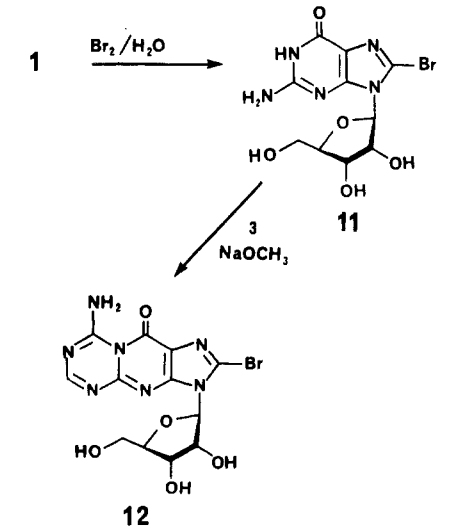
The ^{13}C NMR spectroscopic assignments for IA′-metamorphosine (**2**) were made with the aid of the proton magnetic resonance spectra of the two labeled compounds **5** and **7**. In the proton-coupled ^{13}C NMR spectrum of **7**, the following changes were observed. The resonance at δ 161.57, a doublet in **2** (J = 201.8 Hz), appeared as a broad singlet and the resonances at δ 158.51 and 150.97, doublets in **2** (J = 11.75 and 13.15 Hz, respectively), appeared as singlets, while the remaining four resonances for the N-heterocyclic base moiety were unchanged in multiplicity. Accordingly, the signal at δ 161.57 could be assigned unambiguously to C6 and the signals at δ 158.51 and 150.97 to C8/C4a (Table II).

In the proton-coupled ^{13}C NMR spectrum of **5**, the resonance at 140.0 ppm, a doublet in **2** (J = 215.8 Hz), appeared as a broad singlet, the resonance at δ 119.10, a doublet in **2** (J = 11.42 Hz), appeared as a singlet, and the resonance at 148.96, a multiplet in **2**, appeared as a doublet due to coupling with the C1′-H. The remaining ^{13}C resonances were unaffected. Accordingly, the resonances at δ 140.0, 119.10, and 148.96 could be assigned to C2, C10a, and C3a, respectively. The resonance at δ 157.55, which was unchanged in both of the labeled compounds **5** and **7**, was therefore due to C10. These assignments are summarized in Table II.

The ultraviolet spectrum (EtOH) was indicative of the extended ring system, and the mass spectrum (FAB) showed the expected $M^+ + 1$ and $B^+ + 2$ peaks at m/e 336 and 204, respectively. While the analytical and spectroscopic data were consistent with proposed structure **2**, they were not definitive for distinguishing it from the three other isomeric products (**8**, **9**, **10**) that might have arisen



from the combination of **1** and **3**. Therefore, we sought confirmation of structure **2** by X-ray crystallographic analysis. The 2-bromo derivative of IA′-metamorphosine (**12**) was synthesized from 8-bromoguanosine (**11**) and methyl *N*-cyanomethanimidate (**3**) with sodium methoxide in methanol. As in the case of **2**, there were two distinct N–H signals in the ^{1}H NMR spectrum in $(CD_3)_2SO$, at δ 10.15 and 9.40. The hydrogen on the triazine ring (6-H) appeared at δ 8.09 (Table I). However, the compound did not form crystals suitable for X-ray diffraction. Accordingly, we resorted to a pair of parallel spectroscopic matches involving compound **2** and its 2-bromo derivative (**12**) with the product of 9-benzylguanine[20] plus **3** and its corresponding bromo derivative.



The $C_{14}H_{11}N_7O$ product (**14**) from the reaction of 9-benzylguanine (**13**) and methyl *N*-cyanomethanimidate (**3**) with sodium methoxide in methanol was similar to the guanosine product **2** in UV, ^{13}C NMR, and ^{1}H NMR

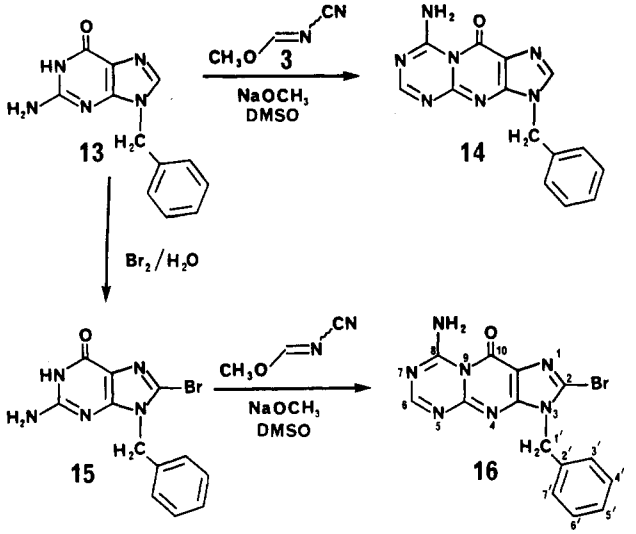
(20) Noell, C. W.; Robins, R. K. *J. Med. Chem.* **1962**, *5*, 558.

Table III. Bond Lengths in Crystalline $C_{14}H_{10}N_7BrO$, 16 (a and b)[a]

type[b]	length, Å	
	molecule a	molecule b
$Br-C_2$	1.876 (4)	1.874 (4)
N_1-C_2	1.289 (6)	1.279 (6)
N_4-C_{4a}	1.313 (6)	1.321 (5)
N_5-C_6	1.297 (6)	1.316 (6)
N_7-C_8	1.316 (6)	1.328 (6)
N_3-C_2	1.367 (6)	1.391 (6)
N_3-C_{3a}	1.382 (5)	1.375 (5)
$N_3-C_{1'}$	1.469 (6)	1.457 (6)
$C_{3a}-C_{10a}$	1.375 (6)	1.370 (6)
$C_{10}-C_{10a}$	1.420 (6)	1.403 (6)
$O-C_{10}$	1.326 (6)	1.226 (6)
N_1-C_{10a}	1.395 (5)	1.397 (5)
N_4-C_{3a}	1.335 (6)	1.352 (6)
N_5-C_{4a}	1.361 (6)	1.351 (6)
N_7-C_6	1.352 (7)	1.329 (6)
N_9-C_{4a}	1.436 (6)	1.435 (6)
N_9-C_8	1.423 (5)	1.402 (5)
N_9-C_{10}	1.449 (5)	1.471 (6)
$N-C_8$	1.314 (6)	1.301 (7)
$N-H_{N1}$	0.88 (4)	0.92 (5)
$N-H_{N2}$	0.83 (4)	0.74 (5)

[a] The numbers in parentheses are the estimated standard deviations in the last significant digit. [b] Atoms are labeled in agreement with structures **4a** and **4b** in ref 1.

spectra for the tricyclic moiety (Table I, II) but did not form crystals satisfactory for X-ray diffraction. The $C_{14}H_{10}BrN_7O$ product (**16**) from the reaction of 9-benzyl-8-bromoguanine (**15**) and **3** was spectroscopically similar to the monobromo derivative of IA′-metamorphosine (**12**) and did provide crystals of X-ray diffraction quality.[1] The structural analogy of **14** to **2** and **16** to **12** is evident from the excellent agreement in spectroscopic data (see Tables I and II for ^1H and ^{13}C NMR chemical shifts and Experimental Section for UV spectra).



crystal forms
a and b

Single-crystal X-ray examination of the product of 9-benzyl-8-bromoguanine (**15**) with **3** proved the structure to be as indicated: 8-amino-3-benzyl-2-bromo-3,10-dihydro-10-oxo-1,3,5-triazino[1,2-*a*]purine (**16**), which also established the structure of the guanosine product as **2**. Accordingly, its appellation as IA′-metamorphosine might be considered appropriate. Two crystallographically independent molecules of **16** (**a** and **b**) were present in the solid-state structure,[1] and the interatomic distances and bond angles are given in Tables III and IV. Both molecules are nearly planar through the entire tricyclic base moiety. The crystallographic data[21] were collected on a computer-controlled Four-Circle Nicolet Autodiffractometer by using ω scan technique and Mo Kα radiation (λ 0.71073 Å). The intensities of 5153 independent reflections were collected of which 2983 reflections having intensities greater than 3.0 times their standard deviation were used in the refinement. The data were corrected for Lorentz and polarization factors, and an empirical absorption correction was also applied. The structure was solved by heavy-atom Patterson techniques. Final R, R_w and "goodness of fit" were 0.037, 0.033 and 1.44, respectively.[21]

All hydrogen atoms which are covalently bonded to carbon atoms were included in the structure factor calculations as idealized atoms (assuming sp^2 or sp^3 hybridization of the carbon atom with a C–H bond length of 0.96 Å) "riding" on their respective carbon atoms. The isotropic thermal parameter of each hydrogen atom was fixed at 1.2 times the equivalent isotropic thermal parameter of the carbon atom to which it is covalently bonded. Hydrogen atoms H_{N1} (toward N7) and H_{N2} (toward 10-O in **16**) were refined as independent isotropic atoms; their isotropic thermal parameters refined to final values of 4 (1) Å^2 and

Table IV. Bond Angles in Crystalline $C_{14}H_{10}N_7BrO$, 16 (a and b)[a]

type[b]	angle, deg	
	molecule a	molecule b
BrC_2N_1	123.9 (3)	126.2 (4)
BrC_2N_3	121.0 (3)	118.7 (3)
$N_1C_2N_3$	115.1 (4)	115.1 (4)
C_8NH_{N1}	108 (3)	116 (4)
C_8NH_{N2}	121 (3)	123 (3)
$H_{N1}NH_{N2}$	131 (4)	120 (5)
$C_2N_1C_{10a}$	103.2 (4)	103.8 (4)
$C_2N_3C_{3a}$	105.4 (4)	103.9 (3)
$C_{3a}N_4C_{4a}$	114.6 (4)	114.5 (4)
$C_{4a}N_5C_6$	117.5 (4)	116.4 (4)
$C_6N_7C_8$	115.4 (4)	115.2 (4)
$C_{4a}N_9C_8$	116.1 (3)	116.7 (3)
$C_{4a}N_9C_{10}$	122.7 (3)	121.5 (3)
$C_8N_9C_{10}$	121.1 (4)	121.8 (4)
$C_2N_3C_{1'}$	128.6 (3)	129.3 (3)
$C_{3a}N_3C_{1'}$	126.1 (4)	126.7 (4)
$N_3C_{3a}N_4$	126.0 (4)	124.9 (4)
$N_3C_{3a}C_{10a}$	105.2 (4)	106.8 (4)
$N_4C_{3a}C_{10a}$	128.8 (4)	128.3 (4)
$N_4C_{4a}N_5$	118.1 (4)	117.4 (4)
$N_4C_{4a}N_9$	122.5 (4)	122.9 (4)
$N_5C_{4a}N_9$	119.4 (4)	119.7 (4)
$N_5C_6N_7$	128.7 (5)	129.4 (4)
NC_8N_7	118.4 (4)	117.5 (4)
NC_8N_9	118.8 (4)	120.0 (4)
$N_7C_8N_9$	122.8 (4)	122.6 (4)
$OC_{10}N_9$	122.4 (4)	121.1 (4)
$OC_{10}C_{10a}$	126.2 (4)	126.6 (4)
$N_9C_{10}C_{10a}$	111.4 (4)	112.2 (4)
$N_1C_{10a}C_{3a}$	111.2 (4)	110.3 (4)
$N_1C_{10a}C_{10}$	128.8 (4)	129.0 (4)
$C_{3a}C_{10a}C_{10}$	119.9 (4)	120.5 (4)

[a] The numbers in parentheses are the estimated standard deviations in the last significant digit. [b] Atoms are labeled in agreement with structures **4a** and **4b** in ref 1.

(21) The crystal structure analysis report was provided by Dr. Cynthia S. Day of Crystalytics Company, Lincoln, NE 68501. Crystal data: $C_{14}H_{10}BrN_7O$, apparent M_r 372.2, triclinic space group $P\bar{1}$-C_i^1 (no. 2), a = 8.542 (4) Å, b = 14.371 (5) Å, c = 11.642 (5) Å, α = 93.68 (3)°, β = 92.74 (3)°, γ = 98.46 (3)°, V = 1408 (1) Å^3, ρ_{calcd} (Z = 4) 1.76 g cm^{-3}, temperature 20 ± 1 °C. The weighting scheme used in the least-squares minimization of the function $\sum w(|F_o| - |F_c|)^2$ is defined as $1/\sigma_F^2$. $R = \sum ||F_o| - |F_c||/\sum |F_o|$, $R_w = [\sum w||F_o| - |F_c||^2/\sum_w |F_o|^2]^{1/2}$. GOF = $\{\sum w(|F_o| - |F_c|)^2/(NO - NV)\}^{1/2}$, where NO is the number of observations and NV is the number of variables.

4 (1) Å^2 for molecule **16a** and 7 (2) Å^2 and 2 (1) Å^2 for molecule **16b**. For both molecules in the crystal, the benzyl group is almost perpendicular to and directly below the plane of the tricyclic heterocycle. Dimensions for the benzyl group, which are not exceptional, are not included in Table III. The complete crystallographic data for 8-amino-3-benzyl-2-bromo-3,10-dihydro-10-oxo-1,3,5-triazino[1,2-*a*]purine (**16a,b**), including tables listing atomic positional and thermal parameters, bond angles, torsional angles, intermolecular contact distances, weighted least-squares planes, and observed and calculated structure factors, have been provided as Supplementary Material with our earlier communication.[1] In amplification of the statement made above describing IA′-metamorphosine as a potential dimensional probe with resemblance to adenosine and *lin*-benzoadenosine,[10,11] the increased width of **2** with respect to adenosine can be expressed in the calculated N9–C10a and C4a–C3a interatomic distances. These are respectively for **16a**: 2.37 and 2.23 Å. For **16b**: 2.39 and 2.25 Å. Thus, the central ring is somewhat wider at the top than at the bottom of the structures pictured.

IA′-Metamorphosine (**2**) exhibits fluorescence on a TLC plate (silica gel); however, in aqueous solution at 20 °C, Φ = only 0.003 (compared with Rhodamine G) for λ_{max}^{ex} 350 nm and λ_{max}^{em} 361–590 nm (broad), τ (by phase) = 0.38 ns. In glycerol at 20 °C, by contrast, Φ = 0.19, λ_{max}^{em} 444 nm, τ = 1.31 ns (by phase), 1.38 ns (by modulation), and at −38 °C, Φ = 0.985, probably due to a change in the facility of proton transfer from NH_2 to the *peri*-carbonyl in the excited state as well as a decrease in energy release via vibrational processes with increasing viscosity and lower temperature.

The reagent described herein, methyl *N*-cyanomethanimidate and NaOMe in methanol, has potential application as a spray reagent for thin-layer or paper chromatography. Observed fluorescence develops on heating a sprayed chromatogram at the location of guanosine and adenosine but not at cytidine or uridine. The absence of fluorescence does not necessarily indicate the absence of conversion by means of the reagent. Since only adenine- or adenosine-containing compounds react with chloroacetaldehyde as a spray reagent to develop fluorescence,[22] the two spray reagents can be used in parallel for full differentiation. Compound **2** can serve also as a "protected" guanosine (**1**) since quantitative reconversion of **2** to **1** occurs upon treatment with 0.1 N NaOH at 20 °C within 5 min.

Since IA′-metamorphosine (**2**) contains outer N-heterocyclic rings analogous to those in adenosine, including a β-D-ribofuranosyl moiety attached to the analogous imidazole nitrogen, we explored the possibility that **2** could function as a fluorescent inhibitor of the deamination of adenosine to inosine by adenosine deaminase.[23] IA′-Metamorphosine (**2**) was found to be a moderately effective competitive inhibitor, K_i = 230 μM, with respect to adenosine, K_m = 33 μM. In the presence of a large amount of enzyme, a slight shift in the absorption spectrum of IA′-metamorphosine occurred. If that change in the spectrum in fact reflected an enzymatic reaction, a turnover number of 6 per molecule of enzyme per min can be estimated. Since the corresponding turnover number of adenosine is 8300, we conclude that **2** is at best a very poor substrate for adenosine deaminase. The compound is nonmutagenic in the bacterial mutagen (Ames) screening test.[24]

In conclusion, we have provided (a) a fluorescent agent (**2**) that may mimic (by activity or inhibition) adenosine, (b) a fluorescent spray reagent for guanosine-containing units, (c) a removable blocking group for guanosine, and (d) a concept based upon specific alteration in the form of a nucleoside (or deoxynucleoside) that can hopefully be extended to other substrates.

Experimental Section

Melting points were determined on a Büchi or a Thomas-Hoover capillary melting point apparatus and are uncorrected. ^{1}H nuclear magnetic resonance spectra were recorded on a Varian EM-390, XL-200, or a Nicolet 360 Fourier transform instrument operating at 90, 200, or 360 MHz, respectively, with tetramethylsilane as an internal standard. ^{13}C NMR spectra were obtained on a Varian XL-200 or a Nicolet 360 Fourier transform instrument operating at 50 or 90 MHz, respectively, and chemical shifts are reported in parts per million from tetramethylsilane. Mass spectra were run on a Varian MAT CH-5 low-resolution spectrometer coupled with a 620i computer and STATOS recorder. The fast atom bombardment (FAB) mass spectra were run on a Varian 731 or 311A instrument. Ultraviolet spectra were obtained on a Beckman Acta MVI spectrophotometer. Medium-pressure liquid chromatographic separation was carried out on a self-assembled instrument at a flow rate of 3.5 mL/min. Fluorescence excitation and emission spectra were measured on a Spex Fluorolog spectrofluorometer. Microanalyses were performed by Josef Nemeth and his staff.

8-Amino-3,10-dihydro-10-oxo-3-β-D-ribofuranosyl-1,3,5-triazino[1,2-*a*]purine (IA′-Metamorphosine) (2). To a stirred suspension of dry guanosine (**1**) (2.4 g, 12 mmol) in anhydrous methanol (150 mL) at 10–15 °C under N_2 was added a solution of sodium methoxide prepared from sodium (600 mg, 26 mmol) and methanol (100 mL). The nearly clear solution so formed was treated with methyl *N*-cyanomethanimidate (7.4 g, 88 mmol) (**3**) introduced through a hypodermic syringe. The bulky, white precipitate which separated initially gradually dissolved again during the next few hours, and stirring was continued for a total period of 24 h. Methanol was evaporated slowly in vacuo at room temperature. The resulting milky white solid that separated was washed with a little dry methanol, collected, and air-dried. The crude product (3.6 g) was dissolved in a minimum quantity of DMF, mixed with Woelm silica gel (6 g) (32–63 μ particle size) and evaporated to dryness on a rotary evaporator at <40 °C. The residue was loaded onto a column of the same silica gel (150 g) and eluted with acetone and then with acetone–methanol (5:1). The fractions containing a single fluorescent compound were pooled and evaporated to obtain IA′-metamorphosine as a white solid (0.76 g). The later fractions which were revealed by TLC to contain a mixture of IA′-metamorphosine and unreacted guanosine were combined and evaporated. Repeated chromatographic separation of the mixture so collected, by following the procedure described above, gave additional quantities of IA′-metamorphosine amounting to a total of 1.56 g (yield 39%, or 66% based on unrecovered guanosine): mp 247–249 °C dec; ^{1}H NMR ($(CD_3)_2SO$) δ 8.3 (s, 1, 2-H), 8.03 (s, 1, 6-H), 5.8 (d, 1, anomeric CH), 5.1–5.5 (br, ribose OH's), 4.5, 4.13, 3.94 (all dd, 1 each, ribose Φ 3.63 (br, 2, CH_2OH), 9.39 and 10.2 (br, 1, NH, each exchangeable Φ D_2O); ^{13}C NMR ($(CD_3)_2SO$) δ 161.57 (C6), 158.51 (C8 or C4a), 157.55 (C10), 150.97 (C4a or C8), 148.96 (C3a), 140.0 (C2), 119.10 (C10a), 87.19 (C1′), 85.71 (C4′), 73.93 (C2′), 70.29 (C3′), 61.29 (C5′); UV (EtOH) λ_{max} (ε) 331 nm (9500), 309 (8700), 253 sh (7200), 245 sh (9800); (pH = ~1) λ_{max} (ε) 326 (7400), 306 (7600), 251 sh (6900), 241 sh (9600); mass spectrum (FAB, glycerol matrix), *m/e* 336 (M^+ + 1), 204 (B^+ + 2); fluorescence (H_2O) λ_{ex} 350 nm, λ_{em} 361–590 nm (broad), Φ = 0.0027, τ = 0.38 ns (by phase); (glycerol at 20 °C) Φ = 0.19, λ_{max}^{em} 444 nm, τ = 1.31 ns (by phase), 1.38 ns (by modulation); (glycerol at −38 °C), Φ = 0.985, λ_{max}^{em} 450 nm. Anal. Calcd for $C_{12}H_{13}N_7O_5$: C, 42.99; H, 3.9; N, 29.25. Found: C, 42.96; H, 3.85; N, 29.01.

[8-^{2}H]Guanosine (4). Guanosine (1.25 g) was dissolved in 15 mL of D_2O by warming and the solution was heated under reflux

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(23) Zielke, C. L.; Suelter, C. H. "The Enzymes", 3rd ed.; Boyer, P. D., Ed.; Academic Press: New York, 1971; Vol. 4, p 47.

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for 7 h. The product which separated as a crystalline solid was collected, washed with water, and recrystallized from water to give 1.15 g (92%) of [8-^{2}H]guanosine. The intensity of the singlet at δ 7.85 in the ^{1}H NMR spectrum due to the imidazole CH was 7% of that of the singlet at δ 5.65 due to the anomeric proton, which corresponded to 93% deuterium incorporation at C8.

[2-^{2}H]-8-Amino-3,10-dihydro-10-oxo-3-β-D-ribofuranosyl-1,3,5-triazino[1,2-*a*]purine ([2-^{2}H]-IA′-Metamorphosine) (5). A solution of [8-^{2}H]guanosine (4) (0.85 g, 3 mmol) in sodium (150 mg, 6.5 mmol) and anhydrous methanol (60 mL), prepared as above, was treated with methyl *N*-cyanomethanimidate (1.85 g, 22 mmol) under N_2 at 10–15 °C for 24 h. The reaction mixture was worked up and the product was separated by column chromatography according to the procedure described above: mp 248–249 °C dec; yield 0.36 g (36%); UV spectrum identical with that of **2**; ^{1}H NMR spectrum ($(CD_3)_2SO$) singlet at δ 8.3 (imidazole CH) <10% of that at δ 8.03 (triazine CH).

Trimethyl [1-^{2}H]Orthoformate.[25] A solution of sodium methoxide was prepared from CH_3OD (49.5 g, 1.5 mol, 99.5 atom % D) and sodium (6.9 g, 0.333 mol) and cooled at 30 °C. To this was added $CDCl_3$ (13.4 g, 0.12 mol) in small portions, and a temperature of 50–60 °C was maintained by immersion of the flask into an ice–water bath when necessary. After complete addition, the mixture was refluxed for 10 min, cooled, and then diluted with anhydrous ether. Sodium chloride was removed by filtration and the product was obtained by distillation through a 50-cm Podbielniak column. The fraction which distilled 90–97 °C was recombined with recovered CH_3OD and utilized in a later reaction. A pure fraction, bp 99–101 °C, was obtained in a 5–6 g yield. The recovered material was added to fresh CH_3OD to make 49.5 g and this was treated as above to provide an additional 5–6 g of pure trimethyl [1-^{2}H]orthoformate. After six cycles and utilization of a total of 100 mL of CH_3OD, the combined yield of product was 33 g, 39% yield based on CH_3OD. Greater than 93% isotopic purity was shown by ^{1}H NMR. Redistillation afforded material of bp 100–100.5 °C.

Methyl [1-^{2}H]-*N*-Cyanomethanimidate (6). In a round-bottomed flask equipped with a Dean–Stark trap and a reflux condenser were placed 11.8 g (0.11 mol) of trimethyl [1-^{2}H]-orthoformate, 4.2 g (0.10 mol) of cyanamide, and 12 mL of cyclohexane. The Dean–Stark trap was filled with cyclohexane and the reaction mixture was refluxed gently under nitrogen with stirring until no more methanol was collected in the trap (~1.5 h). The reaction mixture was cooled and diluted with dry ether. Any solid that separated was removed by filtration. After removal of the solvent on a rotary evaporator, the residual oil was distilled at 65–67 °C (2.5 torr) to give the product as a colorless oil: 7.3 g, 87%; ^{1}H NMR spectrum indicated that the singlet at δ 8.3 normally present for methyl *N*-cyanomethanimidate[1] due to ≥C–H had been greatly reduced, corresponding to 95% substitution by deuterium.

[6-^{2}H]-8-Amino-3,10-dihydro-10-oxo-3-β-D-ribofuranosyl-1,3,5-triazino[1,2-*a*]purine ([6-^{2}H]-IA′-Metamorphosine) (7). To a solution of guanosine (1) (0.85 g, 3 mmol) in a solution of sodium methoxide prepared from sodium (0.15 g, 6.5 mmol) and dry methanol (60 mL) was introduced methyl [1-^{2}H]-*N*-cyanomethanimidate (6) (1.85 g, 22 mmol). The reaction mixture was stirred under N_2 at 10–15 °C for 24 h and subsequently worked up as described for IA′-metamorphosine (2): yield, 0.37 g (37%); mp 248–249 °C dec; UV spectrum identical with that of **2**; ^{1}H NMR ($(CD_3)_2SO$) intensity of singlet at δ 8.03 (triazine CH) <10% of that at δ 8.3 (imidazole CH).

8-Amino-2-bromo-3,10-dihydro-10-oxo-3-β-D-ribofuranosyl-1,3,5-triazino[1,2-*a*]purine (2-Bromo-IA′-metamorphosine) (12). To a stirred solution of sodium methoxide prepared from sodium (0.166 g, 7.2 mmol) and anhydrous methanol (65 mL) was added 8-bromoguanosine (11) (1.27 g, 3.5 mmol). The clear solution thus formed was treated with methyl *N*-cyanomethanimidate (1.47 g, 17.5 mmol) under N_2 at room temperature for 24 h. The reaction mixture was evaporated to dryness on a rotary evaporator. The residual solid (2.5 g) was dissolved in a minimum volume of DMF, mixed with Woelm silica gel (5 g), and evaporated to dryness at <40 °C on a rotary evaporator. The residue was loaded onto a column of the same silica gel and eluted with acetone. The fractions containing a single fluorescent compound were combined and evaporated to obtain 0.5 g (35%) of 2-bromo-IA′-metamorphosine (12): mp 190–230 °C dec; ^{1}H NMR ($(CD_3)_2SO$) δ 8.09 (s, 1, 6-H), 5.79 (d, 1, anomeric CH), 5.52, 5.3 (both d, 1, ribose OH's), 5.1, 5.0 (both dd, 1, ribose CH's), 4.2 (br, 1, ribose CH), 3.96 (br, 1, ribose OH), 3.7, 3.54 (both m, 1, 5′-H's) 9.40 and 10.15 (s, 1, NH, each exchangeable with D_2O); ^{13}C NMR ($(CD_3)_2SO$) δ 162.58 (C6), 158.83 (C8 or C4a), 156.87 (C10), 151.56 (C4a or C8), 150.32 (C3a), 127.06 (C2), 120.34 (C10a), 90.8 (C1′), 87.17 (C4′), 71.79 (C2′), 71.17 (C3′), 62.53 (C5′); UV (EtOH) λ_{max} (ϵ) 336 nm (8580), 313 (7990), 257 sh (10 355), 250 sh (12 130); mass spectrum (FAB, glycerol matrix) 414 ($M^+ + 1$, ^{79}Br), 416 ($M^+ + 1$, ^{81}Br). Anal. Calcd for $C_{12}H_{12}BrN_7O_5 \cdot H_2O$: C, 33.34; H, 3.26; N, 22.68; Br, 18.49. Found: C, 33.27; H, 3.18; N, 22.49; Br, 18.91.

8-Amino-3-benzyl-3,10-dihydro-10-oxo-1,3,5-triazino[1,2-*a*]purine (14). A solution of sodium methoxide prepared from sodium (0.58 g, 25 mmol) and dry methanol (10 mL) was added to a stirred solution of 9-benzylguanine (1.205 g, 5 mmol)[20] in dimethyl sulfoxide (25 mL), and then methyl *N*-cyanomethanimidate (3.36 g, 40 mmol) was introduced through a hypodermic syringe. The reaction mixture was stirred under N_2 at room temperature for 16 h. The solution was cooled below 10 °C by the addition of chopped ice and was then acidified to pH 4–5 with 5% hydrochloric acid. The product that separated as a solid was collected, washed with water, and air-dried (1.6 g). The crude product was dissolved in 50 mL of DMF, mixed with Woelm silica gel (5 g), and evaporated to dryness on a rotary evaporator at <45 °C. The residue was loaded onto a column of the same material and eluted with chloroform–methanol (4:1). The fractions containing a single fluorescent compound were collected and evaporated to dryness to yield 1.3 g of product. An analytically pure sample was obtained by recrystallization from DMF–H_2O (1.17 g, 80%); mp 248 °C dec; ^{1}H NMR ($(CD_3)_2SO$) δ 10.25 (s, 1, NH, exchangeable with D_2O), 9.37 (s, 1, NH, exchangeable with D_2O), 8.23 (s, 1, 2-H), 8.03 (s, 1, 6-H), 7.38 (m, 5, benzene protons), 5.33 (s, 2, CH_2); ^{13}C NMR ($(CD_3)_2SO$) δ 161.42 (C6), 158.65 (C8 or C4a), 157.73 (C10), 150.93 (C4a or C8), 149.03 (C3a), 141.83 (C2), 118.73 (C10a), 46.11 (C1′) (see **16** for benzyl numbering) 136.65 (C2′), 128.67 (C3′ and C7′ or C4′ and C6′), 127.48 (C4′ and C6′ or C3′ and C7′), 127.79 (C5′); UV (EtOH) λ_{max} (ϵ) 334 nm (9825), 312 (9080), 256 sh (7140), 247 (9980); mass spectrum (70 eV), *m/e* (relative intensity) 293 (M^+, 73.6), 91 ($C_7H_7^+$, 100). Anal. Calcd for $C_{14}H_{11}N_7O$: C, 57.33; H, 3.77; N, 33.44. Found: C, 57.26; H, 3.47; N, 33.62.

9-Benzyl-8-bromoguanine (15). A well stirred suspension of 9-benzylguanine (13) (2.41 g, 10 mmol) in water (50 mL) was treated with 1% (v/v) aqueous bromine (150 mL) in portions at room temperature. The reaction mixture was stirred for a further period of 30 min following the addition. The pale-yellow solid that separated was washed with water. Analytically pure sample was obtained by recrystallization from DMF (2.4 g, 75%): mp <300 °C; ^{1}H NMR ($(CD_3)_2SO$) δ 7.1–7.35 (m, 5, phenyl protons), 6.56 (s, 2, NH_2 exchangeable with D_2O), 5.2 (s, 2, CH_2); UV (pH 11) λ_{max} (ϵ) 273 nm (13 410); mass spectrum (70 eV), *m/e* (relative intensity) 319 (M^+ ^{79}Br, 10), 321 (M^+ ^{81}Br, 9), 91 ($C_7H_7^+$, 100). Anal. Calcd for $C_{12}H_{10}BrN_5O$: C, 45.02; H, 3.15; N, 21.88; Br, 24.96. Found: C, 45.45; H, 3.02; N, 22.26; Br, 24.29.

8-Amino-3-benzyl-2-bromo-3,10-dihydro-10-oxo-1,3,5-triazino[1,2-*a*]purine (16). To a stirred solution of 9-benzyl-8-bromoguanine (15) (1.6 g, 5 mmol) in dimethyl sulfoxide (40 mL) (slight warming was necessary), a solution of sodium methoxide prepared from sodium (0.46 g, 20 mmol) and methanol (10 mL) was added. Methyl *N*-cyanomethanimidate (3.32 g, 40 mmol) was then introduced, and the reaction mixture was stirred for 16 h at room temperature under N_2. The resulting solution was cooled below 10 °C by the addition of pieces of ice and was then acidified with 5% hydrochloric acid to pH 4–5. The product that separated as a pale-yellow solid was collected, washed with water, and recrystallized from DMF–H_2O to furnish 1.35 g (73%) of analytically pure sample: mp 280–282 °C dec; ^{1}H NMR ($(CD_3)_2SO$) δ 10.18 (s, 1, NH, exchangeable with D_2O), 9.46 (s, 1, NH, ex-

(25) (a) Baughcum, S. L.; Duerst, R. W.; Rowe, W. F.; Smith, Z.; Wilson, E. B. *J. Am. Chem. Soc.* **1981**, *103*, 6296. (b) Adapted from the published procedure for triethyl orthoformate; see: Kaufman, W. E.; Dreger, E. E. "Organic Syntheses"; Wiley: New York, 1941; Collect. Vol. 1, p 258.

changeable with D_2O), 8.07 (s, 1, 6-H), 7.23-7.38 (m, 5, phenyl protons), 5.32 (s, 2, CH_2); ^{13}C NMR ($(CD_3)_2SO$) δ 163.17 (C6), 159.88 (C8 or C4a), 157.9 (C10), 152.83 (C4a or C8), 151.88 (C3a), 127.76 (C2), 120.35 (C10a), 47.95 (C1') (see **16** for benzyl numbering), 136.83 (C2'), 130.1 (C3' and C7' or C4' and C6'), 129.24 (C4' and C6' or C3' and C7'), 128.43 (C5'); UV (EtOH) λ_{max} (ϵ) 336 nm (8580), 313 (7990), 257 sh (10 355), 250 (12 130); mass spectrum (70 eV), *m/e* (relative intensity) 371 (M^+ ^{79}Br, 10), 373 (M^+ ^{81}Br, 9), 91 ($C_7H_7^+$, 100). Anal. Calcd for $C_{14}H_{10}BrN_7O$: C, 45.18; H, 2.71; N, 26.35; Br, 21.47. Found: C, 45.44; H, 2.73; N, 26.59; Br, 21.35.

Regeneration of Guanosine (1) from IA'-Metamorphosine (2). A solution of 1.1 mg of IA'-metamorphosine (**2**) in 100 mL of 0.1 N NaOH was allowed to stand for 5 min. The UV spectrum showed the complete disappearance of absorption at 331 nm and a new absorption maximum appeared at 264 nm. Quantitative determinations with guanosine as a standard indicated 100% conversion of **2** to **1**.

Spray Reagent of Methyl *N*-Cyanomethanimidate (3). A solution of 1.5 g of **3** and 1 equiv of powdered, anhydrous $NaOCH_3$ in 50 mL of anhydrous methanol was finely sprayed over chromatograms (silica gel) which were spotted with varied amounts of adenosine, guanosine, cytidine, and uridine. The plates were heated for 2-5 min with a heat gun, and blue tinted fluorescent spots became pronounced at adenosine[1] and guanosine. The plates remained dark at cytidine and uridine.

Adenosine Deaminase (Adenosine Aminohydrolase, EC 3.5.4.4) Inhibition by 2. Potassium phosphate buffer, pH 7.45 (50 mM), was used throughout. Substrate solutions were made by dissolving adenosine in buffer and the stock concentrations were 109, 165, 212, 316, and 629 μM. Inhibitor solutions were made by dissolving **2** in buffer, and the stock concentrations were 1.82, 2.78, 4.61, and 6.69 mM. Calf intestinal mucosal adenosine deaminase (10 μL of Grade I commercial solution, Sigma) was suspended in 90 μL of buffer containing 0.1 mg per mL of bovine serum albumin (BSA). This enzyme solution was then diluted 150-fold with 0.1 mg per mL of BSA solution. The final assay mixtures had a total volume of 1.0 mL in a cuvette with a 1.0-cm light path. The assay mixtures contained 0.7 mL of buffer, 0.1 mL of the appropriate stock solution of **2**, 0.1 mL of the adenosine deaminase solution, and 0.1 mL of the appropriate adenosine stock solution. The rates of deamination were determined at 25 °C by monitoring the drop in absorbance at 265 nm. The reference cell in each run contained **2** at the same concentration as in the sample cell. Assays were run in triplicate for each sample. The initial slopes were converted to micromoles per minute per milligram of protein by using 8.1 as the difference in mM extinction coefficient between adenosine and inosine. Subsequent Lineweaver-Burk[26] analysis of the data established that the inhibition was competitive. Analysis with the COMP program[27] gave K_m for adenosine, 32.7 ± 1.9 μM, V_{max} 293 ± 9 μmol/(min mg), and K_i for **2**, 227 ± 10 μM.

Reaction of IA'-Metamorphosphine (2) with Adenosine Deaminase. A solution of IA'-metamorphosine (0.30 μmol in 3 mL of 0.1 M triethylammonium bicarbonate, pH 7) containing adenosine deaminase (80 μg, 2.3 mmol, desalted by passage over a 1-mL column of Sephadex G-10 in 0.1 M triethylammonium bicarbonate) was incubated at room temperature for 5 h. During this period, the absorption spectrum shifted slightly (λ_{max} 318 and 300 nm, clean isosbestic points at 326, 306 and 256 nm); the pH also increased, but its readjustment to pH 7 did not affect the spectrum. Neither the pH nor the spectrum changed in a control without enzyme. Upon overnight incubation, the spectrum of the enzyme-containing sample underwent further change and lost its isosbestic points. Attempts to isolate the product after 3.5 h incubation by lyophilization followed by dissolution of the residue in methanol led to the recovery of a compound with a UV spectrum resembling that of guanosine; compound **2** not exposed to adenosine deaminase was recovered intact. These data suggest that adenosine deaminase did react with IA'-metamorphosine, albeit very slowly, but the failure to isolate and characterize the product precludes further interpretation at this point.

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Annelation of Isocytosines by Reaction with Methyl *N*-Cyanomethanimidate and Sodium Methoxide: Influence of Substitution on the Course of the Reaction and Rearrangements

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Annelation of isocytosine and 6-substituted isocytosines with methyl *N*-cyanomethanimidate and sodium methoxide in methanol leads to pyrimidotriazines. The structures of the products reveal substituent influence on the direction of cyclization and rearrangement. The exocyclic NH_2, newly formed in the cyclization process, may undergo further condensation with methyl *N*-cyanomethanimidate and sodium methoxide in methanol, and this feature of the reaction can be used for selective monomethylation of the exoheterocyclic NH_2. A gentle method for the formation of triflate salts of amines involves treatment of the free bases with trimethylsilyl trifluoromethanesulfonate.

The annelation of guanosine to provide a tricyclic *N*-ribonucleoside having binding sites in the terminal rings similar to those of adenosine was accomplished under mild conditions, namely reaction with methyl *N*-cyanomethanimidate and sodium methoxide in anhydrous methanol at 20 °C.[1,2] Through the use of sodium meth-

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