Synthesis and Pharmacological Evaluation of a Series of Analogues of 1-Methylisoguanosine

Robert T. Bartlett, Alan F. Cook,* Michael J. Holman, Warren W. McComas, Eugene F. Nowoswait, Mohindar S. Poonian,

Chemical Research Department, Hoffmann-La Roche Inc., Nutley, New Jersey 07110

Judy A. Baird-Lambert, Brian A. Baldo,* and John F. Marwood

Roche Research Institute of Marine Pharmacology, Dee Why, N.S.W. Australia 2099. *Received August 25, 1980*

A series of analogues of the pharmacologically active marine natural product 1-methylisoguanosine (1) was evaluated for biological activity in muscle relaxant, cardiovascular, antiinflammatory, and antiallergic tests. Modifications at the 1 position produced the ethyl, *n*-butyl, *n*-octyl, and phenyl derivatives **3–6**, respectively. Substitutions at the 8 position provided the bromo, hydrazino and amino compounds **9–11**. Modifications at the 5′ position yielded the deoxy, iodo, and phosphate derivatives **15**, **13**, and **16**, as well as the cyclic 3′,5′-phosphate **17**. The synthesis of the C-nucleoside analogue **19** was achieved from the β-D-ribofuranosylcarboximidic ester **20**. The acylic analogue **29** and the β-D-arabinofuranosyl derivative **35** were both synthesized by reaction of methyl isocyanate with the appropriately protected aminocyanoimidazole precursors **28** and **32**. 1-Methylxanthosine (**12**), isoguanosine (**7**), and 2-methoxyadenosine (**18**) were also synthesized. At doses up to 100 mg/kg po, the 5′-phosphate **16**, cyclic 3′,5′-phosphate **17**, and the O-methylated analogue 2-methoxyadenosine **18** were active in producing muscle relaxation and hypothermia. These compounds possessed antiallergic activity and produced dose-dependent falls in mean blood pressure and heart rate as did the 1-ethyl (**3**) and 1-*n*-butyl (**4**) analogues. In general, antiinflammatory activity paralleled the other results, except that the cyclic 3′,5′-phosphate **17** was inactive at the dose tested, while the 3,5′-anhydronucleoside **14** was weakly active and displayed antiallergic effects.

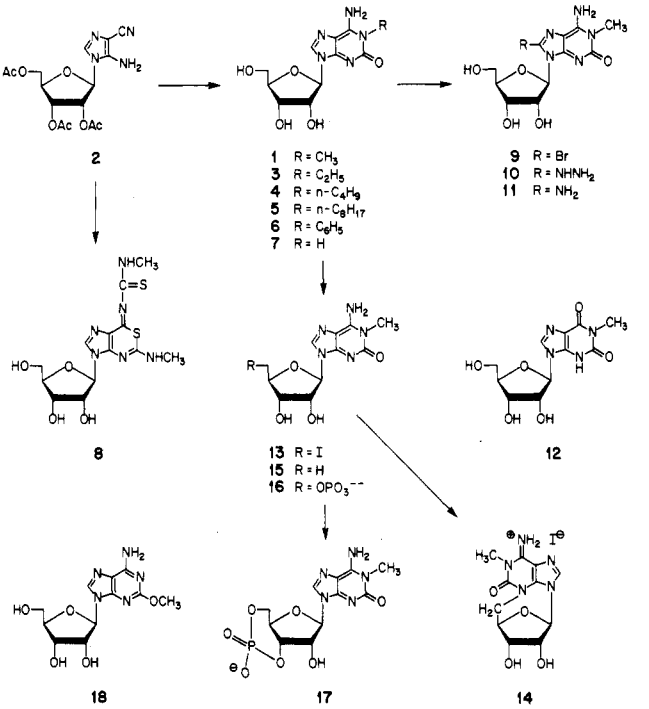
A new methylated purine nucleoside has recently been isolated from the marine sponge *Tedania digitata* and identified by spectral and degradative methods as 1-methylisoguanosine (**1**).[1,2] The isolation of **1** from Californian dorid nudibranches has also recently been reported.[3] The structure of **1** was confirmed by chemical synthesis via two independent methods: (a) cyclization of a protected imidazole nucleoside derived from AICAR and (b) methylation of isoguanosine.[1,2] 1-Methylisoguanosine has been shown to possess potent muscle-relaxant activity, as well as exhibiting cardiovascular and antiinflammatory properties.[3-6] In view of the interest in this compound, a variety of analogues have been synthesized so that the structure–activity relationships in this area could be explored.

Structural variation of 1-methylisoguanosine was accomplished at three general areas of the molecule. Thus, the substituent at N-1 was varied in order to explore the effects of changing the *N*-methyl present in the marine natural product. Functionalization of C-8 was accomplished, and several 8-substituted derivatives of 1-methylisoguanosine were examined. Variation of the N-9 substitution pattern was achieved in two ways by functionalizing the β-D-ribofuranosyl moiety and by replacing the naturally occurring sugar with other groups. Additionally, the effect of O-2 methylation instead of N-1 methylation and the effect of conversion of the N-6 amino to give the keto analogue were examined.

Results and Discussion

The method of synthesis of 1-methylisoguanosine[2] involving reaction of methyl isocyanate with the protected

Scheme I



imidazole nucleoside **2** (Scheme I),[7] followed by cyclization and deprotection using methanolic ammonia, provided direct access to 1-alkyl and 1-phenyl derivatives of isoguanosine by the use of appropriate alkyl and phenyl isocyanates. Although reaction of **2** with ethyl isocyanate or *n*-butyl isocyanate required approximately 8 h for complete consumption of starting material, treatment with *n*-octyl isocyanate required an overnight reaction. In each case, no attempts were made to isolate the intermediate, but the crude mixture was deprotected to give the 1-alkyl derivatives **3**, **4**, or **5** as crystalline solids. Reaction of **2** with phenyl isocyanate proved to be more complex, since a number of unidentified byproducts were produced, even though the reaction was run at room temperature. After

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deprotection, 1-phenylisoguanosine (6) was obtained in relatively poor yield. Isoguanosine (7), also known as crotonoside, was isolated in 1932 from the Croton bean by Cherbuliez and Bernhard[8] and subsequently synthesized by Davoll.[9] Since it was of interest to compare the biological properties of isoguanosine with those of the 1-methyl compound 1, synthesis of 7 was carried out by nitrous acid deamination of 2,6-diamino-9-β-D-ribofuranosylpurine,[10] according to the method of Davoll.[9]

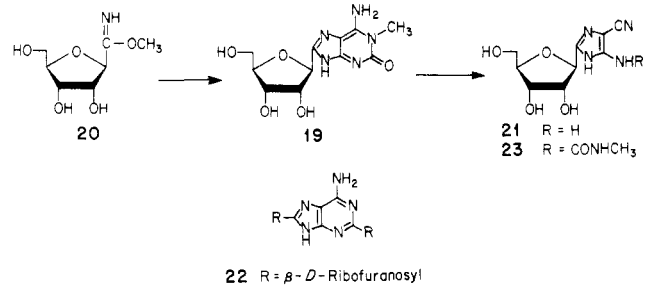
The reaction of the imidazole intermediate 2 with methyl isothiocyanate gave after deprotection a yellow crystalline solid which proved not to be the anticipated 2-thio-1-methylisoguanosine. The NMR spectrum of the product indicated the presence of two compounds in a ratio of approximately 7:3. The spectrum of the major component revealed the presence of overlapping three-proton doublets at δ 2.88 and 2.93, together with exchangeable one-proton doublets at δ 8.45 and 9.35, which indicated the presence of two CH_3NH functionalities. The intense UV absorption of the compound suggested the presence of a highly conjugated chromophore, and the microanalysis indicated the presence of two sulfur atoms in the molecule. On this basis, the compound was designated as the imidazothiazine nucleoside 8, presumably formed by cyclization via the sulfur atom rather than the nitrogen atom of the postulated methylthiocarbamoyl intermediate.

Several 8-substituted derivatives of 1-methylisoguanosine were prepared using 1 as starting material. Reaction of 1 with bromine–water was extremely rapid at room temperature, and the 8-bromo compound 9 was obtained as a relatively insoluble solid in high yield. Attempts to produce the 8-amino compound 11 by reaction of 9 with ammonia were not successful; only starting material was recovered from a reaction with ammonia in methanol, and a mixture of products was obtained by reaction with liquid ammonia at room temperature or at 100 °C. Reaction of 9 with hydrazine, on the other hand, proceeded smoothly, and after reaction at 100 °C for 2.5 h the hydrazino derivative 10 was obtained as a green crystalline solid. This hydrazino compound could be reduced using Raney nickel, with cleavage of the N–N bond and formation of the 8-amino compound 11.

1-Methylxanthosine (12) was considered to be of interest for pharmacological studies, since it can be regarded as the deaminated analogue of 1. Although this compound has previously been synthesized,[11] the route described was considered to be too lengthy for our purposes, and a simpler route was employed. This route involved methylation of guanosine using methyl iodide according to the method of Broom et al.[12] to produce the 1-methyl isomer, which was subsequently deaminated using sodium nitrite in aqueous acid to give 1-methylxanthosine (12).

Iodination of 1 with methyl triphenoxyphosphonium iodide gave the 5′-iodo compound 13. Although this material was completely stable in and could be recrystallized from methanol, it was found to be unstable in water. When an aqueous solution of 13 was heated under reflux, cyclization rapidly occurred to give the 3,5′-anhydronucleoside 14 as the hydriodide salt. Other purine nucleosides have previously been found to undergo cyclizations of this type;[13] adenosine, for example, when treated with methyl triphenoxyphosphonium iodide gave the corresponding 3,5′-anhydronucleoside directly. Similar reactions have been observed in the inosine and guanosine series. The 5′-iodo compound 13 was also reduced using hydrogen and palladium to give the 5′-deoxy compound 15.

Scheme II



In view of the possibility that the actions of 1 in vivo might be mediated via phosphorylated species, such as the 5′-phosphate 16 or the cyclic 3′,5′-phosphate 17, these latter compounds were synthesized for comparison with 1. The 5′-phosphate 16 was prepared by reaction of 1 with phosphoryl chloride in triethyl phosphate,[14] and the crude material was obtained by precipitation of the lithium salt. When this solid was examined by paper chromatography, small amounts of fluorescent material were detected by UV light and further purification was therefore deemed necessary. The crude material was purified by chromatography on a carbon column, and after conversion to the sodium form the pure 5′-phosphate was isolated as a white amorphous solid. The cyclic 3′,5′-phosphate 17 was obtained by reaction of 16 with *N,N′*-dicyclohexylcarbodiimide in pyridine[15] and isolated after chromatography as the sodium salt. Enzymatic experiments indicated that 17 was completely resistant to bacterial alkaline phosphatase and only slightly degraded by the action of snake venom diesterase. Other nucleoside cyclic 3′,5′-phosphates have also been shown to be resistant to these enzymes.[15,16] The 5′-phosphate 16, on the other hand, was completely degraded by bacterial alkaline phosphatase and also by snake venom 5′-nucleotidase, thus confirming the presence of the 5′-phosphate.

2-Methoxyadenosine (18), also known as spongosine,[17] can be considered as an isomer of 1; therefore, it was determined to be of interest for biological evaluation. A convenient synthesis of 18 was achieved by reaction of 2-chloroadenosine[18] with sodium methoxide in methanol under reflux for 18 h.

For the synthesis of the C-nucleoside analogue 19, the versatile C-nucleoside precursor 20[19] was employed (Scheme II).

Reaction of methyl β-D-ribofuranosyl-1-carboximidate (20) with aminomalonitrile *p*-toluenesulfonate[20] in meth-

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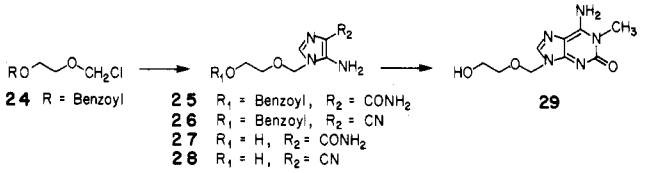
Table I. Pharmacology

no.	ED_{50}, mg/kg po: muscle relaxation	ED_{50}, mg/kg po: hypothermia	cardiovascular responses: mean BP, %	cardiovascular responses: HR, %	dose, mg/kg po	% inhibn: antiinflammatory[a]	% inhibn: anti-allergy[b]
1	11.2 (7.7-16.3)	10.9 (6.3-18.7)	-32	-36	5	62	55
3	>100	>100	-18	-27	50	c (17 at 50 mg/kg)	40
4	>100	>100	-7	-16	100	c	35
5	>100	>100	c	c	100	c	14
6	>100	>100	c	c	100	c	30
7	>100	>100	d	d	d	c	d
9	>100	>100	c	c	100	c	c
10	>100	>100	-13	-21	100	c	c
11	>100	>100	-10	-20	100	c	c
12	>100	>100	c	c	100	c	c
13	>100	>100	c	c	100	c	14
14	>100	>100	c	c	100	c (21 at 50 mg/kg)	45
15	>100	>100	c	c	100	c	c
16	19.7 (9.9-39.2)	12.6 (2.2-71.9)	-44	-48	25	46	57
17	29.5 (24.7-35.2)	21.2 (16.6-26.9)	-17	-37	25	c	14
18	15.3 (8.6-27.1)	12.5 (7.5-20.7)	-41	-25	20	25 (57 at 50 mg/kg)	56
19	>100	>100	-9	c	100	c	c
29	>100	>100	c	c	100	c	c
35	>100	>100	c	c	100	c	c

[a] Tested at a molar equivalent (±10%) of 25 mg/kg 1-methylisoguanosine. [b] 10 mg/kg iv. [c] Indicates no activity at the doses tested. [d] Not tested.

Scheme III



anol in the presence of pyridine gave the cyanoaminoimidazole intermediate **21**. It was found to be important to employ a substantial excess of aminomalonitrile in order to produce acceptable yields of **21**, since with stoichiometric ratios a considerable amount (>50%) of the disubstituted adenine derivative **22** was obtained. Reaction of **21** with methyl isocyanate was investigated in several solvents, and the products were analyzed after cyclization with concentrated ammonia. In contrast to the conditions for the preparation of **1**, methanol was found to be the most useful solvent for the synthesis of **19**. One of the side products isolated from the above reaction was assigned structure **23** on the basis of its IR and NMR spectra. It was thought that **23** might be the immediate precursor of **19**, but in the light of the complete resistance of the former to concentrated ammonium hydroxide that possibility must be ruled out. Further reaction of compound **23** with methyl isocyanate in methanol or dimethylformamide, followed by concentrated ammonium hydroxide treatment at 60 °C, also did not produce **19** but instead gave products of undetermined structures.

The interest in acyclic analogues of purine nucleosides, especially acycloguanosine, an effective, clinically used antiviral agent,[21] prompted our synthesis of the acyclic 1-methylisoguanosine compound **29** (Scheme III). The synthesis of the required imidazole precursor **25** was achieved by a condensation between the chloro ether **24**[22] and aminoimidazolecarboxamide. A number of known condensation procedures were attempted, but the best yield of **25** (15%) was obtained by reaction of sodium aminoimidazolecarboxamide with **24** in dimethylformamide in the presence of triethylamine at room temperature. The site of attachment of the acyclo moiety of **25** was determined by a comparison of the ^{13}C NMR spectrum of its deprotected derivative **27** with that of 5-amino-4-carbamoyl-1-β-D-ribofuranosylimidazole (AICA riboside). A close correspondence between each carbon signal of the heterocycle in the two compounds (AICA riboside, δ 112.02, 128.74, 142.84, and 166.66; compound **27**, δ 112.18, 130.02, 142.86, and 166.15) strongly suggests that the sites of attachment are identical. Conversion of compound **25** into **26** was achieved in 72% yield by the standard phosphorus oxychloride dehydration procedure. The target compound acyclo 1-methylisoguanosine (**29**) was obtained in 60% yield by reaction of **26** with methyl isocyanate in DMF at 60 °C, followed by treatment with concentrated ammonium hydroxide.

For the synthesis of the arabinosyl derivative **35**, the imidazole nucleoside intermediate **31** was required (Scheme IV). The latter was obtained by a condensation of the arabinofuranosyl chloride **30**[23] with aminoimidazolecarboxamide. In order to confirm the structure of **31**, a sample was debenzylated to give the arabinosyl analogue **33**, a compound previously synthesized by Mackenzie and Shaw.[24] The physicochemical properties of **33** were in close agreement with the literature values, thus providing support for the structure of the tribenzyl derivative **31**. In addition, the ^{13}C NMR signals of the heterocyclic moiety of **33** (δ 111.85, 129.24, 142.56, and 166.6) were found to be almost identical with those of AICA riboside. The cyano compound **32** was prepared by a standard procedure, and its conversion into the 1-methylisoguanosine derivative **34** was initially attempted under the same conditions as for **1** or **29**, i.e., methyl isocyanate in either DMF or methanol. Under these conditions very little reaction occurred, and only starting ma-

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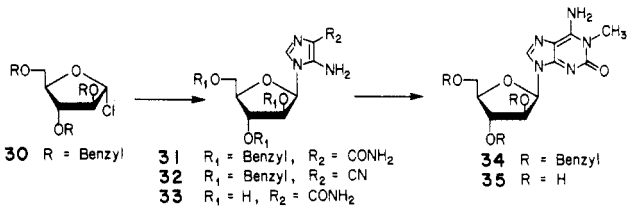
Scheme IV



30 R = Benzyl
31 R_1 = Benzyl, R_2 = $CONH_2$
32 R_1 = Benzyl, R_2 = CN
33 R_1 = H, R_2 = $CONH_2$
34 R = Benzyl
35 R = H

terials were recovered. Reaction of **32** with methyl isocyanate in pyridine under reflux followed by ammonia treatment was more successful, and **34** was produced in a yield of 62%. Palladium-catalyzed hydrogenolysis of **34** in methanol gave a complex mixture which contained a high percentage of non-UV material, together with only small amounts of the desired compound **35**. Debenzylation of **34** could be achieved with boron trichloride at −78 °C, and **35** was obtained in 60–70% yield.

The analogues of 1-methylisoguanosine were examined for their skeletal-muscle relaxant, hypothermic, cardiovascular, and antiinflammatory effects following oral administration and for their antiallergic effects following intravenous administration. The results are summarized in Table I.

After oral administration to mice, 1-methylisoguanosine produced a decrease in muscle tone. This was seen initially as a flaccidity of the hind limbs. With increasing dose, muscle relaxation became more generalized and involved the abdomen and limbs. A concommitant decrease in rectal temperature was also recorded. 1-Methylisoguanosine had an ED_{50} of 11.2 mg/kg for muscle relaxation and 10.9 mg/kg for hypothermia.[4] The analogues were tested up to a dose of 100 mg/kg and only the 5′-phosphate **16** and the cyclic 3′,5′-phosphate **17**, as well as the O-methylated analogue **18**, displayed effects.

1-Methylisoguanosine produced dose-dependent falls in mean blood pressure and heart rate of hypertensive rats. At 5 mg/kg po there was a 32% reduction in blood pressure and a heart rate decrease of 36%.[4] Doses up to 100 mg/kg po were used to evaluate the analogues. The results tended to parallel those obtained for muscle relaxation and hypothermia so that **16–18** had similar effects but at a slightly higher dose (20–25 mg/kg po). Additionally, this test allowed quantitation of the effect of variation of the alkyl substituent at N-1. The 1-ethyl analogue had an effect at 50 mg/kg po, while the 1-*n*-butyl analogue had a weak effect at 100 mg/kg po. Also, weak effects were noted for the 8-substituted analogues, 8-hydrazino-1-methylisoguanosine (**10**) and 8-amino-1-methylisoguanosine (**11**), at 100 mg/kg po.

Inhibition of carrageenan-induced inflammation was observed in rats after oral administration of 1-methylisoguanosine. A 62% inhibition was observed after a dose of 25 mg/kg po, and the analogues were evaluated at a molar equivalent dose (±10%). The antiinflammatory activity of the analogues paralleled the results for muscle relaxant/hypothermia and cardiovascular effects, except that the cyclic 3′,5′-phosphate was inactive at the dose tested and the 3,5′-anhydronucleoside **14** was weakly active producing no inhibition at a dose equivalent to 25 mg/kg of 1-methylisoguanosine but producing 21% inhibition at 50 mg/kg.

The antiallergic effects of the compounds were investigated by examining the inhibition of passive paw anaphylaxis in rats. In this test, 1-methylisoguanosine produced 55% inhibition at 10 mg/kg iv. A number of the analogues exhibited inhibition. From a potential therapeutic viewpoint, it was interesting that the 3,5′-anhydronucleoside **14**, which displayed an antiallergic effect as well as a weak antiinflammatory action, displayed no muscle relaxant/cardiovascular effects. Thus, this compound was investigated further, but it was found that it was inactive as an inhibitor of passive anaphylaxis after oral administration.

The muscle relaxant, hypothermic, and hypotensive effects of the marine natural product 1-methylisoguanosine, as well as a number of the analogues, were accompanied by a decrease in heart rate. None of the analogues investigated had any selectivity of action whereby the bradycardia could be removed or reduced while maintaining the other pharmacological effects. Antiinflammatory properties were present in analogues possessing the other pharmacological effects. However, the 3,5′-anhydronucleoside **14** which displayed antiinflammatory and antiallergic properties did not display muscle-relaxant or cardiovascular effects. **29** and **35** were tested against a herpes virus infection (HSV-1) in mice using a previously outlined procedure.[25] When administered at a dose of 100 mg/kg ip, both compounds were inactive.

Experimental Section

Melting points were determined in a Thomas-Hoover capillary melting point apparatus and are uncorrected. ^{13}C and ^1H NMR spectra were obtained using a Varian XL-100 spectrometer. Unless otherwise stated, Me_2SO-d_6 was used as solvent with tetramethylsilane as internal reference. UV spectra were obtained using a Cary Model 14 recording spectrophotometer. Solvents were dried by storage over molecular sieves (type 4Å), with the exception of methanol which was stored over type 3Å molecular sieves. DMF was dried by distillation over ninhydrin and calcium oxide. Pyridine and triethylamine were purified by refluxing with ninhydrin for 2 h, followed by distillation over KOH pellets; subsequently, these were stored over sieves (4Å).

Substituted Isoguanosines 3–6. A solution of **2** (20 mmol) and the appropriate isocyanate (200 mmol) in DMF (100 mL, dry) was heated with stirring under reflux (bath temperature 100 °C) for 8 h (18 h for **5**; 40 h at 25 °C for **6**) and then evaporated to dryness. The residual oil was treated with concentrated ammonium hydroxide in methanol (200 mL, 1:1) for 18 h and evaporated to dryness, and the residue was recrystallized from water (for **3** and **4**) or methanol (for **5**). During the preparation of **6**, a solid precipitated during the treatment with methanolic ammonia. This solid was removed by filtration and discarded. The filtrate was evaporated to dryness and dissolved in hot ethanol. On storage overnight at 5 °C, a white crystalline solid was deposited, recrystallization of which gave pure **6**.

3: yield 66%; mp 240–241 °C dec; NMR δ 8.09 (br s, 2, NH_2), 7.81 (s, 1, C_8 H), 5.81 (t, CH_2OH), 5.62 (d, 1, J = 7 Hz, $C_{1'}$ H), 5.25 (d, 1, J = 6 Hz, OH), 4.99 (d, 1, J = 4 Hz, OH), 4.56 (m, 1, $C_{3'}$ H), 3.9–4.2 (m, 4, 2 × CH, NCH_2), 3.59 (m, 2, 2 × $C_{5'}$ H), 1.14 (t, 3, J = 7 Hz, CH_3); UV (H_2O) λ_{max} 209–210 nm (ε 25500), 249–250 (8140), 294–295 (11000); UV (0.1 M HCl) λ_{max} 209–210 nm (ε 24150), 236 (5200), 284 (12200). Anal. ($C_{12}H_{17}N_5O_5$) C, H, N.

4 was obtained as the monohydrate: yield 44%; mp 230–231 °C; NMR δ 8.08 (br s, 2, NH_2), 7.86 (s, 1, C_8 H), 5.55–5.75 (m, 2, $C_{1'}$ H, OH), 5.27 (d, 1, J = 6 Hz, OH), 5.03 (d, 1, J = 4 Hz, OH), 4.55 (m, 1, $C_{2'}$ H), 3.8–4.15 (m, 4, $C_{3'}$ H, CH_2N), 3.6 (m, 2, 2 × $C_{5'}$ H), 1.4 (m, 4, CH_2CH_2), 0.88 (t, 3, J = 7 Hz, CH_3); UV (H_2O) λ_{max} 210 nm (ε 26480), 249 (8420), 294 (11580); UV (0.1 M HCl) λ_{max} 210 nm (ε 24850), 235 sh (5580), 283 (13270). Anal. ($C_{14}H_{23}N_5O_6$) C, H, N.

5: yield 33%; mp 191–193 °C; NMR δ 8.08 (br s, 2, NH_2), 7.86 (s, 1, C_8H), 5.60 (m, 2, CH_2OH, $C_{1'}$ H), 5.30 (d, 1, OH), 5.05 (d, 1, OH), 4.52 (m, 1, CH), 3.75–4.1 (m, 4, 2 × CH, NCH_2), 3.53 (m, 2, 2 × $C_{5'}$ H), 1.2 (m, 12, 6 × CH_2), 0.85 (t, 3, J = 6 Hz, CH_3); UV (H_2O) λ_{max} 211 nm (ε 26000), 250 (8250), 295 (11420); UV (0.1 M HCl) λ_{max} 211 nm (ε 24250), 240 sh (5000), 284 (12920). Anal. ($C_{18}H_{29}N_5O_5$) C, H, N.

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(0.1 M HCl) λ_{max} 211 nm (ε 24 250), 240 sh (5000), 284 (12 920). Anal. ($C_{18}H_{29}N_5O_5$) C, H, N.

6: yield 19%; mp 184–200 °C indefinite; NMR δ 7.94 (s, 1, C_8 H), 7.49 (m, 5, NH_2, phenyl), 7.25 (m, 2, phenyl), 5.62 (m, 2, $C_{1'}$ H, CH_2OH), 5.35 (d, 1, J = 7 Hz, OH), 5.09 (d, 1, J = 4 Hz, OH), 4.58 (m, 1, $C_{2'}$ H), 4.09 (m, 1, $C_{3'}$ H), 3.94 (m, 1, $C_{4'}$ H), 3.60 (m, 2, 2 × $C_{5'}$ H); UV (H_2O) λ_{max} 208 nm (ε 33 900), 249 (9100), 296 (12 100); UV (0.1 M HCl) λ_{max} 206 nm (ε 30 100), 284 (14 100), 235 sh (7600). Anal. ($C_{16}H_{17}N_5O_5 \cdot 0.5H_2O$) C, H, N.

1-Methylxanthosine (12). A solution of 1-methylguanosine[12] (7 g) in water (280 mL) was treated with sodium nitrite (14 g) and glacial acetic acid (17 mL) for 60 h at room temperature. The solution was diluted to 1 L with water, partially evaporated to remove dissolved gases, and rediluted to 1 L. This solution was applied to a Carbopack B column (4 × 60 cm, Supelco Inc., Bellefonte, PA), and the column was eluted with water (7 L), followed by ethanol/concentrated ammonium hydroxide/water (7:1:2). The appropriate fractions were combined, evaporated to dryness, and coevaporated with ethanol (2 × 50 mL). The residue was triturated with ethanol to an amorphous solid, which was collected and recrystallized twice from water to give pure **12**: yield 1.3 g (18%); mp 250–280 °C indefinite dec (lit.[11] 225–230 °C dec); NMR δ 7.85 (s, 1, C_8 H), 5.77 (d, 1, J = 6 Hz, $C_{1'}$ H), 5.43 (br s, 1, OH), 5.22 (d, 1, J = 4 Hz, OH), 5.0 (br s, 1, OH), 3.9–4.3 (m, 3, 3 × CH), 3.64 (d, 2, J = 3 Hz, 2 × $C_{5'}$ H), 3.17 (s, 3, CH_3); UV (H_2O) λ_{max} 239 nm (ε 8400), 262 (9580); UV (0.1 M HCl) λ_{max} 238 nm (ε 7700), 262 (9700) [lit.[11] (pH 6) λ_{max} 243 nm (ε 8500), 265 (9800); λ_{max} (pH 1) 239 (7200), 264 (9900)]. Anal. ($C_{11}H_{14}N_4O_6$) C, H, N.

Reaction of 2 with Methyl Isothiocyanate. A solution of **2** (7.3 g) in DMF (100 mL, dry) was treated with methyl isothiocyanate (27 mL) at 100 °C for 44 h. The product was evaporated to a foam, dissolved in chloroform (50 mL), and applied to a silica column (1 kg) which had been packed in chloroform/methanol (25:1). The column was eluted with the same solvent, and fractions 180–240 (20 mL size) were evaporated to dryness. The residue was crystallized from ethyl acetate/hexane to give a yellow crystalline solid (3.3 g). A sample (0.5 g) of this material in methanol (5 mL) was treated with methanolic ammonia (5 mL, saturated) for 24 h and then evaporated to dryness and recrystallized twice from water to give **8**: yield 204 mg (17%); mp 214–217 °C dec; NMR (major isomer) δ 9.35 (d, 1, J = 5 Hz, $NHCH_3$), 8.45 (d, 1, J = 4 Hz, $NHCH_3$), 7.98 (s, 1, CH), 5.79 (d, 1, J = 5 Hz, $C_{1'}$ H), 5.35 (d, 1, J = 6 Hz, OH), 5.08 (d, 1, J = 5 Hz, OH), 4.92 (t, 1, J = 5 Hz, CH_2OH), 4.41 (m, 1, CH), 4.10 (m, 1, CH), 3.90 (m, 1, CH), 3.57 (m, 2, CH_2), 2.93 (d, 3, J = 4 Hz, CH_3NH), 2.88 (d, 3, J = 4 Hz, CH_3NH); UV (H_2O) λ_{max} 391–393 nm (ε 7300), 287 (13 850), 221 (22 600), 205 (24 300); UV (0.1 M HCl) λ_{max} 382–385 nm (ε 6550), 287 (14 020), 208 (22 400). Anal. ($C_{13}H_{18}N_6O_4S_2$) C, H, N, S.

8-Bromo-1-methylisoguanosine (9). Bromine–water (1%, v/v, 114 mL) was added in aliquots of 14 mL to a stirred suspension of **1** (6 g) in water (60 mL) at such a rate that the yellow color of the reaction mixture disappeared between each addition. After the final addition, the suspension was stirred for 15 min and then neutralized with aqueous sodium hydroxide (2 N). After storage overnight, the white solid was collected, washed thoroughly with water, and dried in vacuo to give the 8-bromo compound **9**: yield 6.9 g (91%). A sample was recrystallized from water for analytical purposes: mp 224–230 °C dec; NMR δ 8.29 (s, 2, NH_2), 5.85 (m, 1, CH_2OH), 5.63 (d, 1, J = 7 Hz, $C_{1'}$ H), 5.29 (d, 1, J = 6 Hz, OH), 5.06 (d, 1, J = 5 Hz, OH), 4.97 (m, 1, CH), 4.10 (m, 1, CH), 3.82 (m, 1, CH), 3.56 (m, 2, CH_2), 3.32 (s, 3, CH_3); UV (H_2O) λ_{max} 209 nm (ε 28 650), 257 (11 580), 298 (13 480); UV (0.1 M HCl) λ_{max} 210 nm (ε 26 900), 244 (8980), 289 (15 150). Anal. ($C_{11}H_{14}BrN_5O_5$) C, H, Br, N.

8-Hydrazino-1-methylisoguanosine (10). A suspension of **9** (5 g) in water and aqueous hydrazine (25 mL, 85%) was stirred at 100 °C for 2 h. Since the starting material had not completely dissolved, additional hydrazine (50 mL) was added, and the mixture was stirred at 100 °C for an additional 30 min. The solution was evaporated to dryness and the residue was crystallized twice from water to give **10**, 3.25 g (75%), as green crystals: mp 246–248 °C dec; NMR δ 7.43 (m, 3, NH_2NH), 5.66 (d, 1, J = 7 Hz, $C_{1'}$ H), 5.07 (m, 5, NH_2, 3 × OH), 4.57 (m, 1, $C_{2'}$ H), 4.06 (m, 1, $C_{3'}$H), 3.88 (m, 1, $C_{4'}$ H), 3.58 (m, 2, CH_2), 3.32 (s, 3, CH_3); UV (H_2O) λ_{max} 213 nm (ε 20 400), 255 (10 500), 308 (10 400); UV (0.1 M HCl) λ_{max} 209 nm (ε 20 600), 244 (8350), 298 (10 800). Anal. ($C_{11}H_{17}N_7O_5$) C, H, N.

8-Amino-1-methylisoguanosine (11). A solution of **10** (1.75 g) in water (200 mL) was stirred under reflux in the presence of Raney nickel (17 g, wet weight) for 18 h and filtered through Celite while the solution was still hot. The Celite was washed thoroughly with hot water, and the filtrate and washings were evaporated to dryness and recrystallized from water to give **11**, 724 mg (38%), as the hydrate; mp 242.5–243 °C dec; NMR δ 7.25 (s, 2, NH_2), 6.10 (s, 2, NH_2), 5.92 (br s, 1, OH), 5.65 (d, 1, J = 7 Hz, $C_{1'}$ H), 5.08 (m, 2, 2 × OH), 4.62 (m, 1, $C_{2'}$ H), 4.05 (m, 1, $C_{3'}$ H), 3.88 (br d, 1, J = 2 Hz, $C_{4'}$ H), 3.56 (br s, 2, CH_2), 3.29 (s, 3, CH_3); UV (H_2O) λ_{max} 211 nm (ε 26 200), 255 (12 010), 310 (12 490); UV (0.1 M HCl) λ_{max} 210 (ε 24 710), 246 (9950), 307 (14 410). Anal. ($C_{11}H_{16}N_6O_5 \cdot 2.5H_2O$) C, H, N.

5'-Deoxy-5'-iodo-1-methylisoguanosine (13). A solution of **1** (15 g) in dry DMF (375 mL) was treated with methyl triphenoxyphosphonium iodide (46 g) for 2 h at room temperature. Methanol (60 mL) was added, and after 30 min the solution was evaporated to an oil and partitioned between methylene chloride and 0.02% aqueous sodium thiosulfate (500 mL each). The aqueous layer was washed with methylene chloride (2 × 500 mL), adjusted to pH 6 with aqueous sodium hydroxide, and evaporated to ca. 150 mL. The solution was stored at 5 °C overnight, and the white crystalline solid was collected, washed with water followed by methanol, and dried in vacuo to give the 5'-iodo compound **13**: yield 6.5 g (32%). Recrystallization of a sample from methanol gave analytically pure material: mp 210–215 °C dec; NMR (CD_3OD) δ 7.95 (s, 1, C_8 H), 5.79 (d, 1, J = 5 Hz, $C_{1'}$ H), 4.32 (m, 1, CH), 4.00 (m, 1, CH), 3.54 (m, 5, CH_2, NCH_3); UV (H_2O) λ_{max} 208 nm (ε 27 750), 251 (9680), 294 (12 050); UV (0.1 M HCl) λ_{max} 206 nm (ε 26 550), 236 (6320), 281 (13 400). Anal. ($C_{11}H_{14}IN_5O_4$) C, H, I, N.

3,5'-Anhydro-1-methylisoguanosine Hydriodide (14). A suspension of **13** (1.3 g) in water (70 mL) was heated under reflux with stirring for 70 min, and the solution was evaporated to dryness and recrystallized from water/ethanol to give **14**, 1.08 g (80%), as the monohydrate: mp 235–250 °C dec; NMR δ 8.13 (s, 1, C_8 H), 6.28 (s, 1, $C_{1'}$ H), 5.61 (d, 1, J = 5 Hz, OH), 5.49 (d, 1, J = 6 Hz, OH), 4.6 (m, 2, CH), 4.25 (m, 1, CH), 3.9 (m, 2, CH), 3.44 (s, 3, CH_3); UV (H_2O) λ_{max} 212 nm (ε 9810), 286 (13 470). Anal. ($C_{11}H_{14}IN_5O_4 \cdot H_2O$) C, H, N, I.

5'-Deoxy-1-methylisoguanosine (15). A solution of **13** (500 mg) in methanol (150 mL) containing triethylamine (0.28 mL) was treated with hydrogen at room temperature and pressure in the presence of palladium on carbon (500 mg, 5%), using a Vibromix vibrator for agitation of the solution. After 2 h, the catalyst was removed by filtration through Celite, and the solution was evaporated to dryness. The residual solid was dissolved in hot water (25 mL) and a few drops of 5% aqueous sodium thiosulfate were added to decolorize the solution. On storage overnight at 5 °C, fine needles were deposited. Recrystallization from water gave pure **15**, 150 mg (42%), as the hemihydrate: mp 260–261 °C dec; NMR δ 8.03 (br s, 2, NH_2), 7.85 (s, 1, C_8 H), 5.59 (d, 1, J = 4.5 Hz, $C_{1'}$ H), 5.33 (d, 1, J = 5 Hz, OH), 5.03 (d, 1, J = 5 Hz, OH), 4.47 (m, 1, $C_{2'}$ H), 3.88 (m, 2, $C_{3'}$ H, $C_{4'}$ H), 3.18 (s, 3, NCH_3), 1.27 (d, 3, J = 6 Hz, CH_3CH); UV (H_2O) λ_{max} 209 nm (ε 23 100), 250 (7280), 293 (9960); UV (0.1 M HCl) λ_{max} 208 nm (ε 21 490), 236 (4390), 281 (10 710). Anal. ($C_{11}H_{14}N_5O_4$) C, H, N.

1-Methylisoguanosine 5'-Phosphate (16). Phosphorus oxychloride (4.6 mL) was added dropwise with stirring to a 0 °C suspension of **1** (2.97 g) in dry triethyl phosphate (35 mL). After 2.5 h, the solution was poured with stirring into ice–water (400 mL) and allowed to stand at 5 °C for 3 h. This solution was extracted with ether (3 × 400 mL), adjusted to pH 12 with 10% aqueous lithium hydroxide, and stored at 5 °C overnight. The precipitate of lithium phosphate was removed by filtration through Celite, and the filtrate was neutralized with Dowex 50 (pyridinium form). The resin was filtered off, and the filtrate was evaporated to 100 mL and treated with methanol (300 mL), followed by acetone (1500 mL). The precipitate was collected, washed with acetone, and dried in vacuo to give the crude 5'-phosphate **16**, 3.95 g, as the lithium salt. This material was purified on a Carbopack B column (4 × 60 cm) using a convex gradient of water (2 L) in the mixing vessel) and ethanol/concentrated ammonium

hydroxide/water (5:1:4, 2 L) in the reservoir. The appropriate fractions were combined, evaporated to dryness, converted into the sodium salt, and precipitated from water (15 mL) and methanol (15 mL) by addition of acetone (100 mL) to give **16**, 2.63 g (56%), as the hydrated sodium salt: NMR (D_2O) δ 8.69 (s, 1, C_8H), 6.34 (d, 1, J = 6 Hz, $C_{1'}$ H), 4.99 (m, 1, CH), 4.86 (m, 1, CH), 4.52 (m, 2, CH_2), 3.93 (s, 3, NCH_3); UV (H_2O) λ_{max} 210 nm (ϵ 27 560), 249 (8970), 293 (11 930), UV (0.1 M HCl) λ_{max} 208 nm (ϵ 25 960), 236 (5560), 282 (12 860). Anal. ($C_{11}H_{14}N_5Na_2O_8P\cdot2.5H_2O$) C, H, N, P.

1-Methylisoguanosine Cyclic 3',5'-Phosphate (17). The pyridinium salt of **16** (from 5.46 g of Na salt) and *N,N'*-dicyclohexyl-4-morpholinecarboxamidine (3.74 g, Aldrich) in dry DMF (450 mL) were added dropwise over 5 h to a refluxing solution of *N,N'*-dicyclohexylcarbodiimide (13.8 g) in dry pyridine (1 L). The solution was heated under reflux for a further 18 h and then cooled, evaporated to dryness, treated with water (500 mL), and stored overnight at 5 °C. Solids were removed by filtration through Celite, and the filtrate was neutralized by stirring with an excess of Amberlite IRC-50 resin (hydrogen form). This solution was applied to a column (4 × 64 cm) of DEAE-cellulose (bicarbonate form) and eluted with a linear gradient of triethylammonium bicarbonate, pH 7 (4 L of 0.005 M in the mixing vessel and 4 L of 0.1 M in the reservoir). The appropriate fractions were combined, evaporated to dryness, converted into the sodium salt using Dowex 50 (Na) resin, and precipitated from water (130 mL) and methanol (70 mL) by the addition of acetone (800 mL) to give **17**, 1.5 g (33%), as the sodium salt hemihydrate: NMR δ 8.06 (br s, 2, NH_2), 7.84 (s, 1, C_8 H), 5.71 (s, 1, $C_{1'}$ H), 4.8 (m, 1, CH), 4.38 (d, 1, J = 5 Hz, CH), 4.0 (m, 3, CH, CH_2), 3.35 (s, 3, NCH_3); UV (H_2O) λ_{max} 209 nm (ϵ 25 240), 250 (8380), 293 (10 830); UV (0.1 M HCl) λ_{max} 207 nm (ϵ 25 140), 236 (5580), 281 (12 100). Anal. ($C_{11}H_{13}N_5NaO_7P\cdot0.5H_2O$) C, H, N.

2-Methoxyadenosine (18). A solution of 2-chloroadenosine (6.0 g) in methanolic sodium methoxide (95 mL, 1 mmol/mL) was heated under reflux for 18 h and then cooled and filtered. The filtrate was neutralized and evaporated to dryness, and the residue was recrystalized twice from water to give **18**: yield 3.51 g (59%); mp 189–192 °C (lit.[17] 191–191.5 °C).

5-Amino-4-cyano-2-β-D-ribofuranosylimidazole (21). To a stirred solution of the imidate **20**[19] (9.5 g, 50 mmol) in methanol (60 mL) at 0 °C was added a solution of aminomalonitrile *p*-toluenesulfonate[20] (18.99 g, 75 mmol) in pyridine (75 mL) over a 30-min period. The reaction was stirred for 3 h at ambient temperature, concentrated, and fractionated on a silica gel column (4.5 × 100 cm) which was eluted with acetonitrile/water (95:5). Fractions containing **21** were pooled, concentrated to ca. 50 mL, and acetonitrile was added to turbidity. After storage for 3 days, the solid was collected, washed with ethanol followed by ether, and dried in vacuo to give **21**: yield 5.5 g (46%); mp 187–190 °C; NMR δ 3.55 (m, 2, 5' CH_2), 3.77, 3.99, and 4.05 (m, 1 each, $C_{2'}$ H, $C_{3'}$ H, $C_{4'}$ H), 4.45 (d, 1, $C_{1'}$ H, $J_{1',2'}$ = 4.5 Hz), 4.86 (m, 2, OH), 5.06 (m, 1, OH), 5.93 (br s with shoulder signal at 5.73, 2, NH_2), 11.47 (br s, 1, NH), 12.05 (br s, 1, NH); UV (H_2O) λ_{max} 248–249 nm (ϵ 12 800); IR (KBr) 2210 cm^{-1}. Anal. ($C_9H_{12}N_4O_4$) C, H, N.

For preparation of the side-product **22**, a twofold excess of the imidate **20** was used. This provided a 77.6% yield of **22**, which was recrystallized from water and dried at 100 °C under vacuum: mp 285–287 °C dec; NMR δ 3.4–4.26 (m, 10, 2 × CH_2, 2 × $C_{4'}$ H, 2 × $C_{3'}$ H, and 2 × $C_{2'}$ H), 4.6–5.4 (m, 8, 6 × OH, 2 × $C_{1'}$ H), 7.08 (br s, 2, NH_2), 12.66 (br s, 1, NH); UV (H_2O) λ_{max} 267 nm (ϵ 15 020). Anal. ($C_{15}H_{21}N_5O_8$) C, H, N.

1-Methyl-8-β-D-ribofuranosylisoguanine (19). Methyl isocyanate (4.5 mL) was added to a solution of **21** (3.6 g, 15 mmol) in methanol (100 mL). The reaction was heated at 60 °C and after 1 h additional methyl isocyanate (4.5 mL) was added. After a total of 4 h the reaction was cooled to room temperature and concentrated in vacuo to an oil, which was treated with concentrated ammonium hydroxide for 5 h at 60 °C. The ammoniacal solution was evaporated to a residue, which was stirred with methanol, and the precipitated solid was isolated, washed with methanol, and dried to give crude **19**: yield 2 g (45%). On recrystallization from water, pure **19** was obtained: mp 280–283 °C dec; NMR δ 3.37 (s, 3, N-CH_3), 3.57 (m, 2, CH_2), 3.82 (m, 1, $C_{4'}$ H), 4.0 and 4.13 (both m, 1 each, $C_{2'}$ and $C_{3'}$ H), 4.64 (d, 1, $C_{1'}$ H, $J_{1',2'}$ = 5.5 Hz), 4.8–6.0 (br s, OH and NH), 7.2–8.5 (br s, 2, NH_2); IR (KBr) 1683 and 1648 cm^{-1}; UV (H_2O) λ_{max} 295 nm (ϵ 12 820), 248 (9300), 209 (30 350); UV (0.1 M HCl) λ_{max} 290 nm (ϵ 15 380), sh 235 (ϵ 6700), 206 (ϵ 28 200); UV (0.15 M NH_4OH) λ_{max} 303 nm (ϵ 11 750), sh 248 (5900), 224 (31 580). Anal. ($C_{11}H_{15}N_5O_5$) C, H, N.

The side-product **23** was recrystallized from ethanol: mp 140–142 °C; NMR δ 2.56 (d, 3, $NHCH_3$, J = 4 Hz), 3.75–4.30 (m, 5, $C_{2'}$ H, $C_{3'}$ H, $C_{4'}$ H, 2 × $C_{5'}$ H), 4.43 (d, 1, $C_{1'}$ H, $J_{1',2'}$ = 5 Hz), 5.03 and 5.12 (both m, 1 each, 2 × OH), 5.87 (br, 2, OH and NH), 6.95 (m, 1, $NHCH_3$), 11.51 (br, s, 1, NH); IR (KBr) 2215 cm^{-1}; UV (H_2O) λ_{max} 248 nm (ϵ 12 000). Anal. ($C_{11}H_{15}N_5O_5\cdot0.25H_2O$) C, H, N.

4-Carbamoyl-5-amino-1-[[2-(benzoyloxy)ethoxy]methyl]imidazole (25). Paraformaldehyde (7.18 g) was added to a solution of benzoylethylene glycol (39.76 g, 239.3 mmol) in 1,2-dichloroethane (350 mL, dry). The heterogeneous mixture was cooled to 5 °C and a stream of dry hydrogen chloride was bubbled through the mixture for 3 h. The solution was then allowed to stand over anhydrous calcium chloride overnight, the dessicant was removed, and the solution was concentrated to an oil. The oil was dissolved in dry DMF (100 mL), and the solution was added dropwise to a cooled (0 °C) solution of sodium 4-aminoimidazole-5-carboxamide in DMF (35.28 g, 217 mmol in 100 mL) and triethylamine (40 mL) over a period of 25 min. The reaction was stirred at room temperature for 16 h and then filtered to remove insoluble material. The filtrate was concentrated to an oil, which was dissolved in chloroform, washed with water, and dried over sodium sulfate. The dried organic extract was concentrated and dissolved in ethanol (50 mL). The ethanol solution was treated with activated charcoal and partially evaporated. The crystals were isolated, washed successively with ethanol and diethyl ether, and dried in vacuo to yield **25**: 5.8 g; mp 129–131 °C. The mother liquor was fractionated on a column (2.5 × 45 cm, Merck silica 60, 70–230 mesh), which was eluted with ethyl acetate followed by acetone. The acetone fraction provided a second crop (5.15 g) of the product: total yield of **25** 10.95 g (15%); NMR δ 3.77 and 4.36 (m, 2 each, OCH_2CH_2O), 5.36 (s, 2, OCH_2N), 5.92 (s, 2, NH_2), 6.78 (br, 2, $CONH_2$), 7.29 (s, 1, imidazole CH), 7.4–7.98 (m, 5, phenyl); UV (ethanol) λ_{max} 266 nm (ϵ 1300), 231 (17 900). Anal. ($C_{14}H_{16}N_4O_4$) C, H, N.

4-Carbamoyl-5-amino-1-[(2-hydroxyethoxy)methyl]imidazole (27). The benzoyl precursor **25** (2.6 g, 8.54 mmol) was treated with concentrated ammonium hydroxide (100 mL) at 60 °C for 5 h, the solvent was evaporated, and the residue coevaporated twice with ethanol. The residue was dissolved in methanol (10 mL), and the product was precipitated by the dropwise addition of this solution into diethyl ether (350 mL). The precipitate was recrystallized from 2-propanol to give **27**: yield 1.51 g (88%); mp 130–131 °C; ^1H NMR δ 3.3–3.9 (br, 4, OCH_2CH_2O, partially obscured by D_2O signal), 4.68 (m, 1, OH), 5.25 (s, 2, OCH_2N), 5.83 (s, 2, NH_2), 6.76 (br, 2, $CONH_2$), 7.22 (s, 1, imidazole CH); IR (KBr) 1670 cm^{-1}; UV (H_2O) λ_{max} 265–266 nm (ϵ 11 150), sh 235 (5600); ^{13}C NMR δ 59.76, 69.47, 72.26, 112.18, 130.02, 142.86, 166.41. Anal. ($C_7H_{12}N_4O_3$) C, H, N.

4-Cyano-5-amino-1-[[2-(benzoyloxy)ethoxy]methyl]imidazole (26). A solution of **25** (4.081 g, 13.41 mmol) was treated with freshly distilled phosphorus oxychloride (1.4 mL) in the presence of triethylamine (9 mL) at 5 °C for 17 h. The product which precipitated in the reaction flask was isolated, washed with chloroform, and dried: yield 2.79 g (73%); mp 148–149 °C; NMR δ 3.74 and 4.34 (m, 2 each, OCH_2CH_2), 5.32 (s, 2, OCH_2N), 6.33 (s, 2, NH_2), 7.33 (s, 1, imidazole CH), 7.52–7.98 (m, 5, phenyl); IR (KBr) 2200 cm^{-1}; UV (ethanol) sh 275 nm (ϵ 14 300) λ_{max} 231 (29 800). Anal. ($C_{14}H_{14}N_4O_3$) C, H, N.

4-Cyano-5-amino-1-[(2-hydroxyethoxy)methyl]imidazole (28). A sample of **26** (2.79 g, 9.74 mmol) was heated with concentrated ammonium hydroxide (250 mL) at 60 °C for 5 h. The reaction was concentrated to a residue which was crystallized from ethanol and dried in vacuo at 80 °C to give the crystalline cyanoamino derivative **28**: yield 1.26 g (71%); mp 149–150 °C; NMR δ 3.47 (s, 4, OCH_2CH_2O), 4.64 (br, 1, OH), 5.24 (s, 2, NCH_2O), 6.18 (s, 2, NH_2), 7.25 (s, 1, imidazole CH); IR (KBr) 2220 cm^{-1}; UV (H_2O) λ_{max} 244 nm (ϵ 12 130). Anal. ($C_7H_{10}N_4O_2$) C, H, N.

1-Methyl-9-[(2-hydroxyethoxy)methyl]isoguanine (29). The cyanoaminoimidazole precursor **26** (2.82 g, 9.87 mmol) was dissolved in anhydrous dimethylformamide (75 mL), and methyl

isocyanate (6 mL) was added to the solution. The reaction was heated under reflux (100 °C bath temperature) under anhydrous conditions for 18 h. The solution was cooled, concentrated to an oil, and treated with concentrated ammonium hydroxide (500 mL) at 60 °C for 5 h. The solvent was evaporated, and the residual oil was suspended in methanol. The precipitate was collected (1.41 g, 59.7%, mp 226–227 °C) and recrystallized from water to yield 1.05 g of analytically pure **29**: mp 234–235 °C; NMR δ 3.32 (s, 3, NCH_3), 3.46 (s, 4, OCH_2CH_2O), 4.64 (br, 1, OH), 5.30 (s, 2, OCH_2N), 7.82 (s, 1, imidazole CH), 8.04 (br, 2, NH_2); UV (0.1 N HCl) λ_{max} 282 nm (ϵ 12 180), 235 (5800), 206 (26 500); UV (H_2O) λ_{max} 293 nm (ϵ 11 100), 248 (8400), 208 (28 100); UV (0.15 M NH_4OH) λ_{max} 291 nm (ϵ 10 900), 249 (8280), 208 (27 750). Anal. ($C_9H_{13}N_5O_3$) C, H, N.

1-(2,3,5-Tri-*O*-benzyl-β-D-arabinofuranosyl)-4-carbamoyl-5-aminoimidazole (31). A sample of 2,3,5-tri-*O*-benzyl-1-*O*-*p*-nitrobenzoyl-D-arabinofuranose[23] (11.4 g, 20 mmol) was added to a saturated solution of hydrochloric acid in dichloromethane (30 mL), and the reaction was stirred at 0 °C for 2 h. The precipitate was filtered off and the dichloromethane solution was concentrated in vacuo to an oil, which was dissolved in 40 mL of anhydrous acetonitrile. This solution was treated with 4-amino-5-imidazolecarboxamide hydrochloride (3.24 g, 20 mmol), followed by anhydrous triethylamine (5.6 mL), and heated under reflux on a steam bath for 1 h. The reaction was cooled and evaporated to dryness to give a syrupy residue. The residue was taken up in dichloromethane (75 mL), and the solution was washed with 5% aqueous sodium bicarbonate (2 × 25 mL) followed by water (2 × 25 mL). The organic extract was dried over anhydrous sodium sulfate and concentrated to an oil, which was fractionated on a silica column (Merck prepacked size C) using $CHCl_3/C_2H_5OH$ (25:1) as eluent. The appropriate fractions were pooled and evaporated to give **31**, 1.35 g (13%), as a foam. Compound **31**: NMR δ 3.65 (m, 2, $C_{5'}$ H), 3.9–4.25 (m, 3, $C_{2'}$ H, $C_{3'}$ H), 4.25–4.40 (m, 2, benzyl CH_2), 4.52 and 4.58 (s, 2 each, benzyl CH_2), 5.88 (br, 2, $CONH_2$), 5.97 (d, 1, $C_{1'}$ H, $J_{1',2'}$ = 4 Hz), 6.59 (br, 2, NH_2) 7.15 (s, 1, imidazole CH), 7.29 (m, 15, 3 × phenyl). Anal. ($C_{30}H_{32}N_4O_5$) C, H, N.

1-(2,3,5-Tri-*O*-benzyl-β-D-arabinofuranosyl)-4-cyano-5-aminoimidazole (32). A solution of **31** (15 g, 28.4 mmol) in chloroform (225 mL) and triethylamine (22 mL) was cooled to 0 °C in an ice bath. Phosphorus oxychloride (2.92 mL) was added dropwise with stirring to the cooled solution over ca. 15 min and the stirring continued for 4 h at 0 °C. The reaction was poured into ice (200 g) and the organic layer was separated. The aqueous layer was extracted with chloroform (100 mL), and the combined organic layers were washed with water and dried over anhydrous sodium sulfate. The solution was concentrated to ca. 10 mL and fractionated on a silica column (4 × 100 cm, EM Merck silica gel 60) using $CHCl_3$/EtOH (12:1, v/v) as eluant. Fractions containing the product were concentrated to give **32** as a semisolid: yield 8.96 g (62%); NMR δ 3.62 (m, 2, $C_{5'}$ H), 3.9–4.4 (m, 3, $C_{2'}$ H, $C_{3'}$ H, $C_{4'}$ H), 4.40–4.60 (m, 6, 3 × benzyl CH_2), 5.99 (d, 1, $C_{1'}$ H, $J_{1',2'}$ = 5 Hz), 6.31 (br, 2, NH_2), 7.0–7.4 (m, 16, 3 × phenyl and imidazole CH); IR (KBr) 2220 cm^{-1} (C≡N). Anal. ($C_{30}H_{30}N_4O_4$) C, H, N.

9-(2,3,5-Tri-*O*-benzyl-β-D-arabinofuranosyl)-1-methylisoguanine (34). A solution of the cyanoaminoimidazole derivative **32** (8.7 g, 17 mmol) and methyl isocyanate (120 mL) in anhydrous pyridine (200 mL) was heated under reflux (oil bath temperature 100 °C) for 3 h. The reaction was cooled, evaporated to dryness, and coevaporated with methanol. This syrup was dissolved in methanol (375 mL), treated with concentrated ammonium hydroxide (250 mL), and heated at 60 °C for 3 h in a sealed flask. The solution was cooled, concentrated to a gum, dissolved in chloroform (20 mL), and loaded onto a silica column (4 × 100 cm). The column was eluted with chloroform/ethanol (25:1) and, after evaporation of the appropriate fractions, **34** was obtained as a waxy solid: 6 g (62%); NMR δ 3.37 (s, 3, NCH_3), 3.68 (m, 2, $C_{5'}$ H), 4.0–4.4 (m, 3, $C_{2'}$ H, $C_{3'}$ H, $C_{4'}$ H), 4.52 (s, 2, benzyl CH_2), 4.64 (two overlapping singlets, 4, 2 × benzyl CH_2), 6.18 (d, 1, $C_{1'}$ H, $J_{1',2'}$ = 5 Hz), 7.0–7.4 (m, 15, 3 × phenyl), 7.69 (s, 1, imidazole CH), 8.08 (br, 2, NH_2). Anal. ($C_{32}H_{33}N_5O_5 \cdot 0.5H_2O$) C, H, N.

9-β-D-Arabinofuranosyl-1-methylisoguanine (35). In a dry nitrogen atmosphere the protected precursor **34** (6 g, 10.4 mmol) was dissolved in anhydrous dichloromethane (30 mL) and cooled to −78 °C in a dry ice–acetone bath. To this was added a cooled (−78 °C) solution (42 mL) of 1 M boron trichloride in dichloromethane. The reaction was continued at −78 °C with stirring for 22 h, then allowed to warm to about 0 °C, and added gradually to a slurry of Dowex 1 × 8 (OH^-) resin (150 g) in ethanol (50 mL) so that the pH remained above 6. The resin was filtered off and washed with hot water (2 L), and the filtrate and washings were combined and evaporated to ca. 500 mL. On storage at 5 °C, crystals of **35**, 1.63 g (52%), were deposited, mp 252–253 °C dec. A second crop, 0.48 g (15.3%), of less pure material was also obtained: NMR δ 3.35 (s, 3, NCH_3), 3.6–4.2 (m, 5, $C_{2'}$ H, $C_{3'}$ H, $C_{4'}$ H, $C_{5'}$ H), 5.05 (br, 1, OH), 5.39 (br, 1, OH), 5.58 (br, 1, OH), 5.95 (d, 1, $C_{1'}$ H, $J_{1',2'}$ = 4 Hz), 7.74 (s, 1, imidazole CH), 7.98 (br, 2, NH_2); UV (0.1 M HCl) λ_{max} 209 nm (ϵ 25 100), 234 (5220), 281 (12 450); UV (pH 5) λ_{max} 210 nm (ϵ 25 450), 248 (7920), 292 (11 050); UV (0.15 M NH_4OH) λ_{max} 208 nm (ϵ 24 300), 249 (8000), 292 (10 900). Anal. ($C_{11}H_{15}N_5O_5$) C, H, N.

5-Amino-4-carbamoyl-1-β-D-arabinofuranosylimidazole (33). A solution of the benzylated precursor **31** (0.75 g, 1.41 mmol) in dichloromethane (8 mL) was treated with boron trichloride in dichloromethane (1 M, 6.36 mL) at −78 °C and stirred for 64 h at this temperature. The reaction was quenched with ethanol (10 mL) and neutralized with Dowex 1-X8 (OH^-) resin. The resin was filtered off and washed successively with ethanol and hot water, and the filtrate and washings were concentrated to ca. 5 mL and allowed to crystallize. Crystals of boric acid were removed, and the filtrate was concentrated to an oil which was fractionated on a silica column (Merck size A). The fractions containing the desired product were pooled and evaporated to dryness, and the residue was recrystallized from methanol to give **33**: yield 90 mg (25%); mp 187–188 °C (lit.[24] 191 °C); NMR δ 3.65 (m, 3, $C_{4'}$ H, $C_{5'}$ H), 4.05 (m, 2, $C_{2'}$ H, $C_{3'}$ H), 5.0 (m, 1, OH), 5.45 and 5.53 (m, 2, 2 × OH), 5.75 and 5.77 (m, 3, NH_2, $C_{1'}$ H), 6.67 (br, 2, $CONH_2$), 7.30 (s, 1, imidazole CH); ^{13}C NMR δ 83.46 (C_1), 75.47 and 74.66 ($C_{2'}$, $C_{3'}$), 83.46 ($C_{4'}$), 60.41 ($C_{5'}$), 129.24 (C_2), 111.85 (C_4), 142.56 (C_5), 166.60 (C=O). Anal. ($C_9H_{14}N_4O_5$) C, H, N.

Muscle Relaxation and Hypothermia. Male Füllinsdorf mice weighing 20–25 g were used. Groups of five mice were acclimatized in white plastic boxes with sawdust bedding for approximately 1 h prior to any drug treatment. Room temperature was maintained at 22–24 °C. Compounds were administered orally (po) either dissolved in distilled water or suspended with Tween 80 (2% of the final volume). Muscle relaxation was assessed subjectively on an arbitrary scale of 0–2, where 0 = normal, 0.5 = abnormal hind limb placing, 1 = pronounced hind limb flaccidity without apparent abdominal relaxation, 1.5 = hind limb and abdominal relaxation, 2 = pronounced general flaccidity with little or no spontaneous locomotor activity.

Rectal temperatures were measured immediately after assessment of muscle relaxation. A YSI thermistor probe (Model K23) was inserted approximately 1 cm into the rectum and temperature was recorded on a YSI Telethermometer (Yellow Springs Instrument Co., Yellow Springs, OH). Mean temperature compared with that of a vehicle-treated control group at the same time. Linear regression analysis was used to calculate ED_{50} values, which were the doses required to produce a response equivalent to a half-maximal response of 1-methylisoguanosine.

Cardiovascular Measurements. Young male Füllinsdorf rats (4–5 weeks of age 100–120 g weight range) were injected with 100 mg/kg deoxycorticosterone acetate intramuscularly and maintained on 1% sodium chloride (w/v) drinking solution. Blood pressure rose appreciably within 4 weeks.[26] Blood pressure was measured in conscious hypertensive rats (weight range 220–270 g) after a left carotid arterial catheter was implanted under pentobarbitone sodium (60 mg/kg, ip) anesthesia and exteriorized through the skin at the back of the neck. A jugular venous catheter for administration of test compounds was similarly implanted. Blood pressure was monitored on a Gould Brush Mk 250 pen recorder using a Bell and Howell Model 4-327I pressure transducer. Heart rates were determined from the blood pressure pulse.

Rat Paw Edema Test for Antiinflammatory Activity. Antiinflammatory activity was assessed by the inhibition of carrageenan-induced edema.[27]

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The preparations to be evaluated were administered orally to groups of six male Füllinsdorf rats of body weight range 160–200 g after the animals had fasted overnight. Compounds were dissolved or suspended in water by mixing in a mortar with 1 or 2 drops of Tween 80. Rats were dosed on a milligram per kilogram basis 1 h before measuring the thickness of both hind paws and the subplantar injection of 0.05 mL of carrageenan (Kraft Foods, lot 3F1.11), 1% w/v, in physiological saline. Three hours later the ensuing swelling was measured, and the percent inhibition of the edema formation was calculated by considering the edema in the control animals to be 100%. Paw thickness was measured using the anvil and pin AASE Antiinflammatory Screening Equipment (Roche, Basle) thickness-measuring transducer.

Passive Paw Anaphylaxis Test for Inhibitors of Immediate Hypersensitivity.[28] Sera containing heat-labile homocytotropic antibodies for use in passive paw anaphylaxis (PPA) were raised in 150–200 g female brown Norway rats. Each rat was injected intraperitoneally with 0.5 mL of *Bordetella pertussis* vaccine (Commonwealth Serum Laboratories, Melbourne) containing 2×10^{10} organisms and intramuscularly with 2 mg of crystalline ovalbumin (OA) (Sigma, St. Louis, Grade VI, lot 67C-8035) in 0.5 mL of physiological saline.[29] Eleven days later, animals were bled and sera were recovered and tested for activity in passive cutaneous anaphylaxis (PCA) experiments in rats.[30] For PPA studies, groups of six to eight Oxford Hooded [HO (PVG/C)] rats of approximately 200 g were used. Following the measurement of the thickness of both hind paws, rats were given subplantar injections in each paw of 0.05 mL of a suitable dilution or rat anti-OA serum. Two hours later, animals were injected iv in the tail with 0.3 mL of a 1% w/v solution of OA in physiological saline. Fifteen minutes after antigen challenge, paw swelling was measured using the AASE thickness-measuring transducer. Paws of control animals were injected with diluted antiserum, but these rats were not challenged with antigen before paw thicknesses were read. The mean increase in paw thickness in the antigen-challenged rats was obtained by deducting the mean figure obtained from control animals sensitized with diluted antiserum. Three other groups of control animals used in PPA studies were injected in the hind paws with saline, serum from immunized rats, or serum from rats injected with OA and *B. pertussis* but which gave a PCA titre of 0. Percent inhibition of the immediate allergic reaction was calculated by considering the swelling in the control paws to be 100%. Compounds tested for inhibition of PPA were given iv 5 min before antigen challenge.

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Inhibition of Separated Forms of Cyclic Nucleotide Phosphodiesterase from Pig Coronary Arteries by 1,3-Disubstituted and 1,3,8-Trisubstituted Xanthines

Jack N. Wells,* John E. Garst,[1] and George L. Kramer

Department of Pharmacology, School of Medicine, Vanderbilt University, Nashville, Tennessee 37232.
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A series of xanthines with varied substituents in the 1, 3, and 8 positions were prepared in an attempt to understand the structure–activity relationship for alkylxanthines as inhibitors of two different forms of cyclic nucleotide phosphodiesterase. Polar substituents on the 1 or 3 position of the xanthine reduced the potency of the xanthines to inhibit both the calmodulin-sensitive and the "cyclic AMP specific" forms of phosphodiesterase. Polar substituents on the 8 position of the xanthine, other than a carboxylic acid, increased the potency to inhibit the calmodulin-sensitive form of phosphodiesterase, if they were capable of donating electrons to the xanthine nucleus. On the other hand, any substituent in the 8 position larger than H reduced the potency of the xanthines to inhibit the cyclic AMP specific form of phosphodiesterase. Topographical maps of the active sites of the two forms of phosphodiesterase are presented in summary.

Alkylxanthines (theophylline, caffeine, and 1-methyl-3-isobutylxanthine) are well known as inhibitors of cyclic nucleotide phosphodiesterase activity. We have demonstrated that modification of the xanthine structure can give rise to compounds that inhibit relatively selectively one of the two forms of phosphodiesterase found in pig coronary arteries (peak I and peak II).[2,3] These enzymes appear to be representative of two of the major forms of phosphodiesterase, the calmodulin-sensitive form (peak I) and the "cyclic AMP specific" form (peak II), both of which are found in most mammalian cells. The cyclic AMP specific form is characterized by its relative specificity for cyclic AMP as substrate and by its apparent negative cooperativity. The activity of this form of phosphodiesterase is not altered by calmodulin. The calmodulin-sensitive form has a lower apparent K_m for cyclic GMP (~1–3 μM) than for cyclic AMP (~50–100 μM). The V_{max} of this form of phosphodiesterase with cyclic AMP as substrate, however, is about 2-fold greater than is the V_{max} with cyclic GMP as substrate. Because of the lower K_m for cyclic GMP, this enzyme has been considered to be the cyclic GMP phosphodiesterase; indeed, this form seems to be almost solely responsible for cyclic GMP hydrolysis in pig coronary arteries. That is not to say, however, that the calmodulin-sensitive enzyme can not participate in the regulation of intracellular levels of cyclic AMP. At least in pig coronary arteries, total cyclic AMP phosphodiesterase activity at 1 μM substrate concentration is about equally divided between the calmo-

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