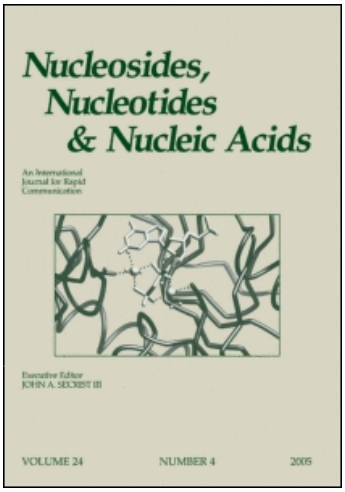

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Thiopurine Nucleosides: Variations in Hydrophobicity Among N[1] Suhstituents

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THIOPURINE NUCLEOSIDES: VARIATIONS IN HYDROPHOBICITY AMONG N^1 SUBSTITUENTS

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Abstract: Syntheses of several 1-substituted 6-thioinosine and 6-thioguanosine derivatives are described. Substituents were selected to provide a range of steric and hydrophobic properties, ranging from amino to benzyl. Proton NMR and mass spectral data are described and discussed.

INTRODUCTION.

The chemistry and biological activity of 6-thiopurines and their derivatives have been of interest for more than four decades. Ready access to the ribonucleosides 6-thioinosine (**1a**) and 6-thioguanosine (**2a**) was realized when Fox and his colleagues discovered that suitably protected guanosine and inosine derivatives could be directly thiated using P_4S_{10} in pyridine.[1] A huge array of biological and clinical studies on the thiopurines, their nucleosides and their nucleotides have appeared and have been reviewed.[2,3]

Montgomery reported in 1963[4] that 1-substituted nucleosides could not be thiated using the pyridine/P_4S_{10} conditions, presumably because the 6-oxo function could not enolize, and that significant debenzylation occurred when the reaction was attempted using protected 1-benyzlinosine. It was subsequently observed that direct thiation of 1-methyl-6-oxo nucleosides could be effected using a higher-boiling solvent (3-picoline) for a shorter period of time.[5] However, these vigorous conditions gave modest yields and led to O-debenzylation when benzyloxy groups were present.

A significant improvement was introduced by Ueda,[6] who discovered that 1-methyladenosine was smoothly converted to 1-methyl-6-thioinosine (**1b**) in reasonable yield by hydrogen sulfide in aqueous pyridine at 60°C. This milder approach appeared to be generalizable to nucleosides having sensitive functional groups. It also appeared that Lawesson's reagent, a remarkably powerful thiating compound,[7] might be suitable for certain of the desired syntheses.

The object of the study reported here was the synthesis of a series of 1-substituted derivatives of 6-thioinosine (s^6I) in which the substituents cover a wide range of hydrophobicities. In addition to the known 1-methyl derivative, the targets were the amino (**1c**), ethyl (**1d**), and benzyl (**1e**) compounds. The synthesis of 1-amino-6-thioguanosine (**2b**) will also be described.

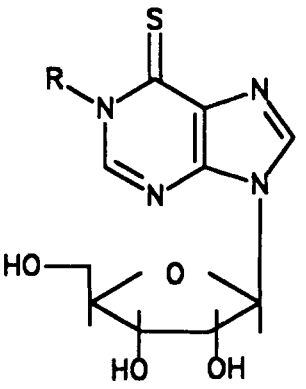
RESULTS AND DISCUSSION

Chemistry. The synthesis of 1-methyl-6-thioinosine (m^1s^6I, **1b**) was carried out as described by Ueda[6], except that the scale was 15 times larger than that reported. The requirement for 230 mL of liquid H_2S mandated a workup in which the stainless steel bomb required for the reaction was vented slowly into a large excess of aqueous NaOH rather than into the atmosphere. The yield of this scaled-up reaction was 71% compared to 57% originally reported on a one gram scale.

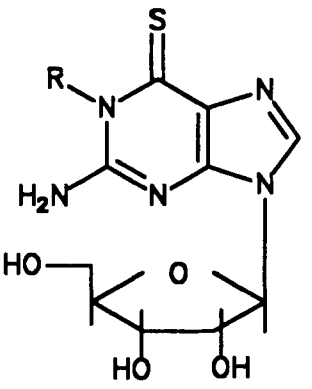
Two approaches to the synthesis of 1-amino-6-thioinosine (am^1s^6I, **1c**) were considered. Direct thiation of 1-aminoinosine[8] was rejected on the basis that the usual thiophosphoric anhydride thiating agents would almost surely react with the free amino function and protection/deprotection strategies designed to circumvent this problem would be tedious. The sulfohydrolysis of 1-aminoadenosine hydrochloride, prepared as previously described[9], proceeded smoothly to give **1c** in 68% yield.

The success of this procedure encouraged its application to the preparation of the 1-ethyl derivative **1d**. Application of the Jones and Robins alkylation procedure[10] as reported by Hanessian[11] required a two-week reaction time for the ethyl iodide alkylation of adenosine. It is not surprising that ethylations of this type proceed much more slowly than methylations, given the greater steric demand of the former[12]. It is quite interesting, however, that all attempts to carry out the sulfohydrolysis reaction gave rise only to N^6-ethyladenosine. The only base in this reaction mixture is pyridine; it came as a considerable surprise that only the Dimroth rearrangement product could be obtained.

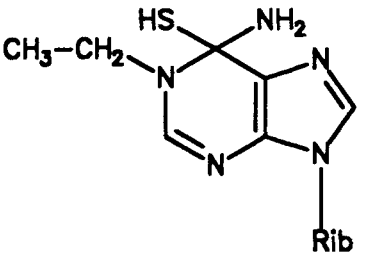
A possible explanation for the dramatic difference in reaction course may involve energies of the transition states along the path to the initial tetrahedral intermediates (**3a, 3b**) which must be formed in these reactions. Wolfenden has described the behavior of 1-methyladenosine both with regard



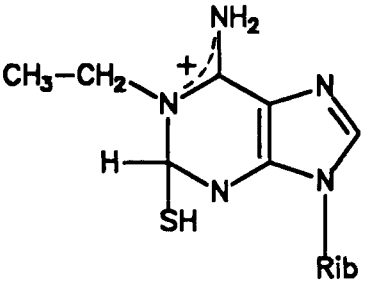
	R
1a	H
b	CH_3
c	NH_2
d	CH_2CH_3
e	$CH_2\phi$



	R
2a	H
b	NH_2



3a



3b

to the ring opening-recyclization characteristic of the Dimroth rearrangement and the nucleophilic attack of hydride at C6.[13] The ethyl group is known to have a significantly greater steric demand than methyl in solvolysis reactions which also proceed via tetrahedral intermediates.[14] As one considers the transition states leading to **3a** and **3b**, it seems likely that the bulkier ethyl group will create greater steric crowding in the former than the latter, since **3b** has a proton on the newly forming tetrahedral center rather than an amino group. Steric crowding might disfavor the transition state leading to **3a** when R is ethyl. Once ring opening has occurred from intermediate **3b**, the reaction will proceed irreversibly to the thermodynamically more stable N^6-ethyladenosine.

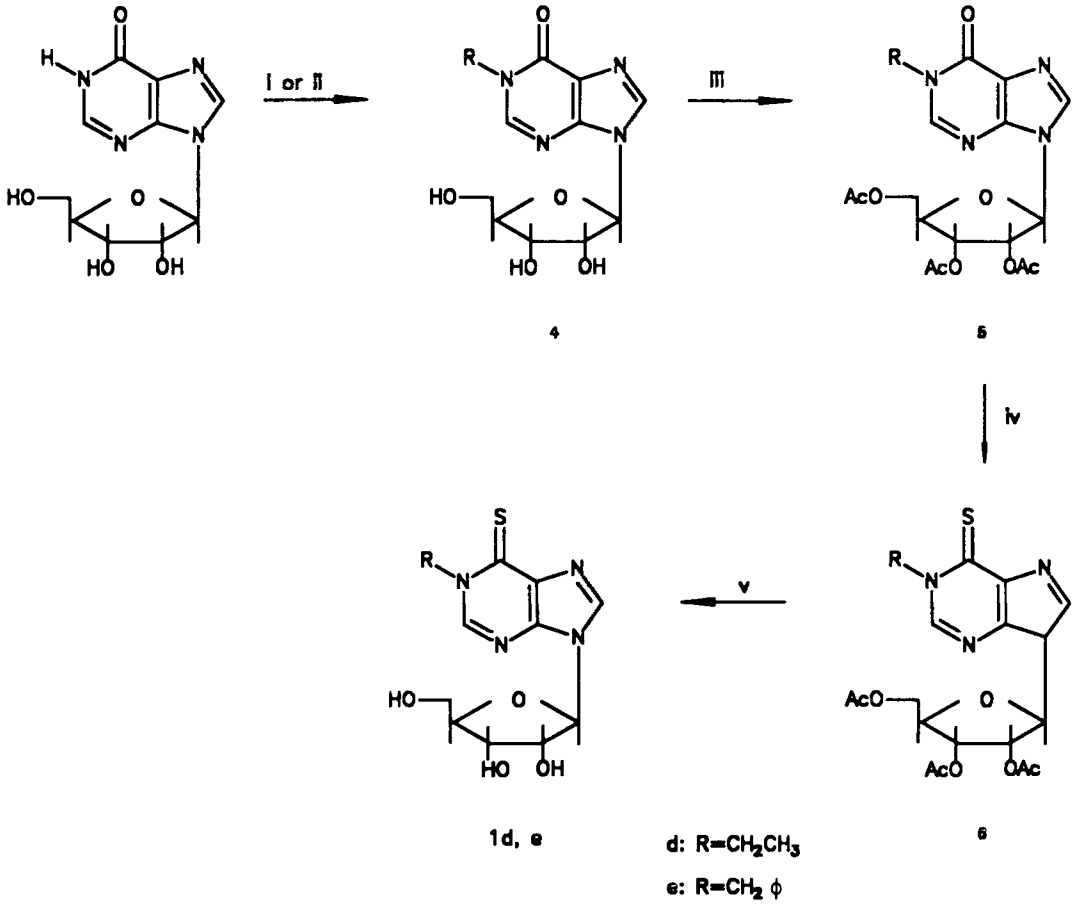
In any case, it was clear that an alternative route was required. The anion of inosine, generated by DBU in dimethyl acetamide, was alkylated using ethyl iodide (Scheme 1). The crude 1-ethylinosine (**4d**), which could not be crystallized, was acetylated to give the protected nucleoside in 72% yield for the two steps. Lawesson's reagent smoothly converted tri-O-acetyl-1-ethylinosine (**5d**) to its 6-thio derivative **6d**, which on treatment with methanolic ammonia gave **1d**. A similar sequence gave 1-benzyl-6-thioinosine (**1e**) in 55% overall yield from inosine.

Finally, the sulfohydrolysis procedure was applied (Scheme 2) to the synthesis of 1-amino-6-thioguanosine (**2b**). The requisite 2-aminoadenosine[15] was prepared from 2-amino-6-chloro-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-purine[16,17] and aminated as described above to give 1,2-diaminoadenosine hydrochloride (**7**). Sulfohydrolysis of **7** gave **2b** in 65% yield.

NMR CONSIDERATIONS

As illustrated in Table 1, it was possible to assign chemical shifts to all of the protons in these nucleosides. Even the sugar hydroxyl groups and other exchangeable protons were fully resolved in dry $(CD_3)_2SO$; all the assignments were unambiguously made using COSY experiments (data not shown). The "thiation shift" which had been previously observed[5] was present in all these molecules; that is, comparing 6-thio with 6-oxo derivatives, the signals for H2 are downfield about 0.4 ppm and for H8 about 0.2 ppm for the former group of compounds. Protons on atoms bound directly to N1 undergo an even greater shift; for example, the N-methylene group chemical shift of 1-ethylinosine appears upfield 0.6 ppm from the corresponding signal for the 6-thio derivative. This effect falls off rapidly with distance, however, and is only 0.09 ppm for the methyl group of the N1-ethyl substituent.

SCHEME 1



I· CH_3CH_2I, DBU, DMAC; II· ϕCH_2Cl, DBU, DMAC;
III· acetic anhydride, pyridine; iv· Lawesson's
reagent, 1,2—dimethoxyethane, reflux; v· methanolic ammonia

It might be noted that for the four s^6I derivatives examined, the chemical shift values for H8 are very close (Table 1). The same is true for H2 except for the benzyl derivative, for which the H2 signal appears 0.12-0.15 ppm downfield from the others. This may reflect either a "contact" effect[8] arising from the greater steric requirements of the benzyl group, or from

TABLE 1[a]

	ϕ	CH_2	H_2	H_8	H_1'	2′-OH	3′-OH	5′-OH	H_2'	H_3'	H_4'	$H_{5',5''}$	NH_2	CH_3
1b			8.80	8.53	5.89	5.54	5.24	5.07	4.49	4.14	3.95	3.62		3.33
1c			8.80	8.58	5.90	5.56	5.24	5.07	4.49	4.15	3.96	3.62	6.83	
1d		4.63	8.83	8.56	5.90	5.50	5.25	5.06	4.51	4.16	3.97	3.66		1.36
1e	7.31	5.87	8.95	8.57	5.91	5.56	5.25	5.08	4.51	4.16	3.97	3.66		
4d		4.03	8.41	8.37	5.87	5.51	5.24	5.09	4.52	4.14	3.96	3.61		1.27
4e	7.33	5.22	8.64	8.38	5.88	5.51	5.20	5.05	4.48	4.13	3.95	3.66		

[a]Proton chemical shifts of selected nucleosides in $(CD_3)_2SO$ expressed as δ(downfield) from internal tetramethylsilane

an average positioning of the benzene ring such that H2 is in the anisotropic deshielding region. In any case, it is clear the benzyl group assumes an average conformation in which the 2-proton is not within the shielding cone of the benzene ring.

It is typically the case that 6-substituted nucleosides give rise to signals for H8 which are downfield from those for H2; indeed, the first exception to that generalization was reported for 1-methylinosine.[18] In order to confirm that the present assignments were correct, advantage was taken of the susceptibility of H8 to exchange with deuterium upon heating in D_2O.[19] Heating a solution of **1b** in D_2O for 20 minutes at 90°C led to a 55% decrease in the intensity of the signal at δ8.32 with no change in the H2 signal at 8.53 or any of the other peaks in the spectrum, confirming the assignments.

MASS SPECTRAL AND HPLC CONSIDERATIONS

The compounds described above were all submitted for mass spectral confirmation of molecular weight. Fast atom bombardment mass spectrometry (FAB/MS) gave a molecular ion (MH^+) and the ion for the base after sugar cleavage (BH^+). Closer examination of the spectra of the N-amino derivatives **1c** and **7** revealed the presence of ions corresponding to the loss of 15 mass units from both the molecular ion and the base ion.

The most obvious reason for such a finding would be contamination by the nonaminated nucleoside. Although quaternization at N1 is a prerequisite for rapid sulfohydrolysis, it has been shown that a very slow conversion of adenosine to 6-thioinosine may occur under forcing conditions.[6] If a small amount of 6-thioinosine were present in **1c**, and if it were to be distributed in the FAB matrix in such a way as to be favorably targeted by the ionizing beam, then it might well show up in the spectrum with an intensity out of proportion to its actual concentration; note that the compounds submitted for MS analysis were all pure by combustion analysis, NMR and tlc.

In order to test this possibility, an HPLC system was developed which gave baseline separation of 6-thioinosine and its 1-amino derivative. This system, which relied on a reversed-phase analytical column and a pH 5 buffer (50 mM KH_2PO_4) containing 15% methanol, revealed that **1c** was about 99.9% pure, but there was detectable about 0.1% of 6-thioinosine.

While it seemed quite unlikely that so small a contaminant could give rise to significant signal in the mass spectrometer, it also seemed that N-N bond cleavage with the loss of the elements of NH would constitute very unusual ion chemistry. The sample was, therefore, subjected to liquid chro-

matography-mass spectrometry (LC-MS). An ammonium acetate solvent system at pH 6[20] gave baseline separation of 6-thioinosine (elution time 16.7 min), 1-amino-6-thioinosine (18.9 min) and 1-methyl-6-thioinosine (22.7 min, used as a control). Mass spectra of the pure components were taken using the thermospray technique[21] and, again, the spectrum of 1c clearly showed the loss of 15 from both MH^+ and BH^+. Neither 6-thioinosine nor the 1-methyl derivative showed similar behavior, ruling out the possibility of odd ion chemistry associated with the ring system itself. The basis for this phenomenon is not understood and will be the object of further study. It does, however, mandate a cautionary note for those who work with N-amino compounds; the appearance of an ion corresponding to the loss of 15 amu does not necessarily imply that the sample is impure.

One final note is in order. In the reverse phase, aqueous buffer systems used for these separations, 6-thioinosine was always eluted prior to the 1-amino derivative, despite the fact that the amino group is considered to be more polar than the proton.[22] This undoubtedly arises from the tendency toward ionization of 6-thioinosine, which has a pKa of about 7.7.[1,23] In support of this conjecture is the fact elution times are much closer together relative to the 1-methyl derivative at pH 5 than at pH 6.

EXPERIMENTAL

Anhydrous solvents were obtained by distilling the commercially-available solvents over CaH_2 under a dry nitrogen atmosphere, with the exception of 1,2-dimethoxyethane (DME), which was used as purchased from Aldrich. The ^{1}H-NMR spectra were recorded on an IBM AF 200 MHz FT-NMR spectrometer. NMR spectra were taken using $(CD_3)_2SO$ or $CDCl_3$ as the solvent and tetramethylsilane (TMS) as the internal standard. The UV spectra were recorded on a Beckman DU-8 spectrophotometer. Low resolution fast atom bombardment (FAB) mass spectra were obtained on a MAT 731 spectrometer using either a glycerol or p-nitrobenzyl alcohol matrix. A Vestec 201 thermospray ionization instrument with a Supelcosil LC-18-S column was used for all LC/MS experiments. The LC solvent system was essentially that of Buck[20] with a flow rate of 2 mL/min. Melting points were determined on a Thomas Hoover Uni-melt capillary melting point apparatus and were uncorrected. Column chromatography for purification purposes involved Merck silica gel grade 60. Elemental analyses were performed by Desert Analytics, Tucson, Arizona. All the intermediates and final thionucleosides were dried under vacuum over P_2O_5.

1-Amino-6-thioinosine (1c)

1-Aminoadenosine hydrochloride[9] (4.78 g, 15 mmol) dissolved in H_2O (43 mL) was placed in a stainless steel bomb and frozen in a dry ice/2-propanol bath. Freshly distilled pyridine (32 mL) was added followed by liquid H_2S (110 mL). The bomb was sealed and heated at 65°C (water bath) for 65 hours. The bomb was vented into aqueous NaOH. The mixture was filtered and H_2O was added to the filtrate to precipitate the product. The product was further purified by recrystallization from a mixture of H_2O and EtOH to give 3 g of **1c**. Yield 68%; mp 204-206°C; UV λ_{max} (ϵ_{max}); pH 1, 319 nm (18.6×10^3); pH 7, 317 (21.6×10^3); pH 11, 317 (21.2×10^3); FAB/MS $(MH)^+$; obsd 300; (MH^+-NH), 285; (BH^+), 168; (BH^+-NH), 153. Anal calcd for $C_{10}H_{13}N_5O_4S.H_2O$: C, 37.8; H, 4.73; N, 22.1; S, 10.1. Found: C, 37.72; H, 4.74; N, 22.11; S, 9.60.

1-Ethylinosine (4d)

Inosine (4.028 g, 1 mmol) was dissolved in a solution of DMAC (22 mL) and DBU (2.5 mL, 16.5 mmol) by stirring at room temperature for 10 minutes. Ethyl bromide (3.4 mL, 45 mmol) was slowly added to the solution. The reaction mixture was stirred at room temperature for 6 hours; it was then poured into a solution of diethyl ether and hexane (500 mL, 4:1, v/v) to precipitate the product. After cooling the mixture at -20°C overnight, the supernate was decanted. The oily residue obtained was dissolved in a minimum volume of EtOH and reprecipitated as before giving a crude oil (4 g,~90% yield) which resisted crystallization. This oil was used in the preparation of **4d** without further purification; UV λ_{max} (ϵ_{max}): pH 1, 250 nm (6.9×10^3); pH 7, 249 (7.5×10^3); pH 11, 249 (6.9×10^3); FAB/MS $(MH)^+$, obsd 297, $C_{12}H_{17}N_4O_5$ requires 297.

2',3',5'-Tri-O-acetyl-1-ethylinosine (5d)

Compound **4d** (4 g, 13.5 mmol) was dried by azeotropic removal of water with dry pyridine. The residue was dissolved in dry pyridine (120 mL) and acetic anhydride (17 mL) was added to the solution. The flask was protected from moisture and the reaction mixture was stirred at 14°C for 48 hours. It was concentrated *in vacuo* to approximately one-third volume and poured into H_2O (100 mL). The aqueous solution was extracted with $CHCl_3$ (4 x 60 mL) and the combined organic extract was washed with a saturated aqueous solution of $NaHCO_3$. The $CHCl_3$ layer was separated and dried over $MgSO_4$. After filtration, the filtrate was concentrated under reduced pressure to give the

crude product, which was further purified by silica gel chromatography using diethyl ether and ethyl acetate (3:1) as the eluent. Yield was 4.5 g (80%). An analytical sample was prepared by cooling a warm solution of **5d** in 2-propanol. The resulting glass was triturated with ether and dried to an analytically pure foam. ^{1}H-NMR ($CDCl_3$); δ8.02 (s, 1H, H2), 7.89 (s, 1H, H8), 6.04 (d, 1H, H1′), 5.83 (t, 1H, H2′), 5.56 (t, 1H, H3′), 4.31 (m, 3H, H4′+H5′+H5′′), 4.06 (q, 2H, $N\underline{CH_2}CH_3$, 2.1 (3s, 9H, $3\underline{CH_3}CO$), 1.33 (t, 3H, $\underline{CH_3}CH_2$); UV λ_{max} (ϵ_{max}) $CHCl_3$; 272 nm (4.4×10^3), 252 (7.2×10^3); FAB/MS $(MH)^+$, obsd 423, $C_{18}H_{23}N_4O_8$ requires 423. Anal calcd for $C_{18}H_{22}N_4O_8 \cdot 0.25\ H_2O$: C, 50.64; H, 5.31; N, 13.12. Found: C, 50.83; H, 5.22; N, 13.00.

2′,3′,5′-Tri-O-acetyl-1-ethyl-6-thioinosine (6d)

Compound **5d** (4.5 g, 10.6 mmol) was dissolved in anhydrous DME after repeated evaporation from the same solvent (3 x 10 mL). Lawesson's reagent (3.7 g, 9 mmol) was added to the solution. The suspension was refluxed for 3 hours under a dry N_2 atmosphere. The reaction mixture was worked up and further purified as described for **6e**. Yield: 3.06 g (65%); ^{1}H-NMR ($CDCl_3$); δ8.25 (s, 1H, H2), 8.01 (s, 1H, H8), 6.04 (d, 1H, H1′), 5.81 (t, 1H, H2′), 5.51 (t, 1H, H3′), 4.59 (q, 2H, $\underline{CH_2}CH_3$), 4.34 (m, 3H, H4′+H5′+H5′′), 2.04 (3s, 9H, $3\underline{CH_3}CO$), 1.42 (t, 3H, $\underline{CH_3}CH_2$); UV λ_{max} (ϵ_{max}) $CHCl_3$; 324 nm (16.8×10^3); low resolution FAB/MS $(MH)^+$ obsd 439, $C_{18}H_{23}N_4O_7S$ requires 439. Anal calcd for $C_{18}H_{22}N_4O_7S \cdot 0.50\ H_2O$: C, 48.3; H, 5.14; N, 12.5; S, 7.15. Found: C, 48.45; H, 4.88; N, 12.27; S, 7.02.

1-Ethyl-6-thioinosine (1d)

Compound **6d** (3.06 g, 7 mmol) was treated with methanolic ammonia solution (saturated at -10°C) (60 mL) for 18 hours at room temperature. The pressure bottle was vented and the contents were concentrated *in vacuo* to an oily residue. The residue was dissolved in hot water and applied to a 2.5 x 44 cm Amberlite XAD-4 column. After washing the column with H_2O (500 mL), the desired product was eluted by switching to a 30% EtOH solution in H_2O. The eluent was evaporated under reduced pressure to give a residue which was redissolved in hot H_2O and lyophilized to yield 1.85 g of solid (85%); UV λ_{max} (ϵ_{max}); pH 1, 323 nm (16.9×10^3), 230 (9.8×10^3); pH 7, 320 nm (20.8×10^3), 231 (9.1×10^3); pH 11, 320 (20.6×10^3), 231 (9.1×10^3); FAB/MS $(MH)^+$ obsd 313, $C_{12}H_{17}N_4O_4S$ requires 313. Anal calcd for $C_{12}H_{16}N_4O_4S$: C, 46.1; H, 5.12; N, 17.9; S, 10.25. Found: C, 45.87; H, 5.05; N, 17.51; S, 10.26.

1-Benzylinosine (4e)[4]

Inosine (4.026 g, 15 mmol) was suspended in a solution of DMAC (22 mL), and 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU, 2.5 mL, 16.5 mmol). The above mixture was stirred for 15 minutes to dissolve the nucleoside. Benzyl chloride (5.2 mL, 45 mmol) was slowly added. The resulting solution was stirred for an additional 5 hours. The solvent was removed *in vacuo* and the residue was dissolved in EtOH. Diethyl ether (400 mL) was added to the ethanolic solution to precipitate the product. After cooling the mixture at -20°C overnight, the supernate was decanted; the yellowish gum was reprecipitated as before. The oily residue obtained was recrystallized from MeOH. White crystals collected by filtration were washed with diethyl ether and MeOH. Yield 5.1 g (95%); mp 209-211°C; UV λ_{max} (ϵ_{max}); pH 1, 255 nm (18.0×10^3); pH 7, 251 (15×10^3); pH 11, 251 (14×10^3); FAB/ MS $(MH)^+$, obsd 359, $C_{17}H_{19}N_4O_5$ requires 359. Anal calcd for $C_{17}H_{18}N_4O_5$: C, 56.9; H, 5.02; N, 15.6. Found: C, 56.59; H, 5.25; N, 15.48.

2',3',5'-Tri-O-acetyl-1-benzylinosine (5e)

Dry **4e** (5.017 g, 14 mmol) was suspended in a solution of dry N,N-dimethyl formamide (DMF, 11.1 mL), dry pyridine (4.2 mL) and acetic anhydride (8.4 mL). The mixture was heated at 75°C for 4 hours, then concentrated *in vacuo* to approximately one-half volume. The concentrate was poured into an ice/H_2O mixture (100 mL). The aqueous solution was extracted with $CHCl_3$ (6 x 40 mL). The combined extracts were washed with a saturated aqueous solution of $NaHCO_3$ (2 x 75 mL) and dried over anhydrous $MgSO_4$. The dried organic extract was filtered and the solvent was removed under reduced pressure to give the crude residue. It was purified on a silica gel column using ethyl acetate and diethyl ether (1:3) as the eluent to afford 6.17 g of compound **5e**. Yield: 6.17 g (91%); ^{1}H-NMR ($CDCl_3$) δ8.04 (s, 1H, H2), 7.91 (s, 1H, H8), 7.35 (m, 5H, phenyl), 6.06 (d, 1H, H1', J=4.9 Hz), 5.88 (t, 1H, H2'), 5.61 (t, 1H, H3'), 5.26 (s, 2H, Bz $\underline{CH_2}$), 4.37 (m, 3H, H4'+H5'+H5''), 2.13 (s, 3H, $\underline{CH_3}CO$), 2.09 (s, 6H, $2\underline{CH_3}CO$) ppm; UV λ_{max} (ϵ_{max}) $CHCl_3$; 273 nm (5.7×10^3), 253 (9.0×10^3); FAB/MS $(MH)^+$, obsd 485, $C_{23}H_{25}N_4O_8$ requires 485. Anal calcd for $C_{23}H_{24}N_4O_8$. 0.50 H_2O: C, 55.9; H, 5.07; N, 11.30. Found: C, 56.04; H, 5.13; N, 11.08.

2',3',5'-Tri-O-acetyl-1-benzyl-6-thioinosine (6e)

A suspension of dry **5e** (6.006 g, 12 mmol) and Lawesson's reagent[7] (2.98 g, 7.4 mmol) in anhydrous DME was refluxed under a dry N_2 atmosphere. After

3 hours of reflux, it was allowed to cool and was poured into H_2O (100 mL). The milky aqueous solution was extracted with $CHCl_3$ (6 x 40 mL). The emulsion formed could be dispersed by the addition of brine. The combined organic extract was once more washed with brine (100 mL). The organic layer was collected and dried over $MgSO_4$. The dried extract was concentrated *in vacuo* to give the crude product. It was purified by silica gel chromatography using diethyl ether and ethyl acetate (3:1, v/v) as the eluent to give 4.25 g of pure **6e**. Yield: 71%. An analytical sample was prepared by recrystallization from isopropanol; mp 70-72°C; ^{1}H-NMR ($CDCl_3$) δ8.25 (s, 1H, H2), 8.05 (s, 1H, H8), 7.36 (m, 5H, phenyl), 6.07 (d, 1H, H1′); 5.86 (t, 3H, H2′+Bz $\underline{CH_2}$), 5.57 (t, 1H, H3′), 4.38 (m, 3H, H4′+H5′+H5′′), 2.13 (s, 3H, $\underline{CH_3}CO$), 2.09 (s, 6H $\underline{CH_3}CO$) ppm; UV λ_{max} (ϵ_{max}) $CHCl_3$; 324 nm (17.1 x 10^3); FAB/MS $(MH)^+$, obsd 501, $C_{23}H_{25}N_4O_7S$ requires 501. Anal calcd for $C_{23}H_{24}N_4O_7S$. 0.50 H_2O: C, 54.21; H, 4.94; N, 10.99; S, 6.29. Found: C, 54.31; H, 4.88; N, 10.90; S, 6.46.

1-Benzyl-6-thioinosine (1e)

Compound **6e** (4.25 g, 8.5 mmol) was treated with methanolic ammonia saturated at -10°C) (70 mL) and the mixture was allowed to stand at room temperature for 18 hours. The pressurized ammonia inside the vessel was released by venting to a methanol trap. The contents were transferred to a beaker and let stand. The white fluffy crystals were collected by filtration and washed with CH_3OH. Yield 2.86 g (90%). An analytical sample was prepared by recrystallization from CH_3OH; mp 124-126°C with softening at 116-188°C; UV λ_{max} (ϵ_{max}); pH 1, 324 nm (18.4 x 10^3); pH 7, 322 (21.6 x 10^3); pH 11, 322 (23.8 x 10^3); FAB/MS $(MH)^+$, obsd 375, $C_{17}H_{19}N_4O_4S$ requires 375. Anal calcd for $C_{17}H_{18}N_4O_4S$: C, 54.5; H, 4.81; N, 14.96; S, 8.55. Found: C, 54.37; H, 4.99; N, 14.61; S, 8.17.

1,2-Diaminoadenosine Hydrochloride (7)

A suspension of powdered 2-aminoadenosine[15] (4.23 g, 15 mmol) and O-(2,4-dinitrophenyl)hydroxylamine[24] (4 g, 20 mmol) was heated at 37°C for 48 hours. The red solution was concentrated *in vacuo* to about one-third of the original volume. After acidification to pH 1 with 0.1 N HCl (15 mL), the mixture was extracted with diethyl ether (4 x 40 mL) to remove the *p*-nitrophenol byproduct of the reaction. The aqueous extract was treated with enough diethyl ether to see a distinct double layer. To it was added suf-

ficient quantity of EtOH to achieve a cloud point and the resulting mixture was refrigerated. The precipitate was filtered and washed with diethyl ether. Yield: 2.76 g (55%). An analytical sample was prepared by chromatography on a 2.5 x 20 cm column of Amerlite XAD-4 using H_2O as the eluent. The fractions containing the produce were pooled and lyophilized to afford pure **7**. ^{1}H-NMR $(CD_3)_2SO$; δ9.58 (bs, 1H, NH), 8.88 (bs, 1H, NH), 8.32 (s, 1H, H8), 8.04 (s, 2H, 2-NH_2), 5.88 (s, 2H, 1-NH_2), 5.74 (d, 1H, H1′), 5.54 (bs, 1H, OH), 5.21 (bs, 2H, two OH), 4.44 (t, 1H, H2′), 4.13 (t, 1H, H3′), 3.92 (t, 1H, H4′), 3.58 (m, 2H, H5′+H5′′) ppm; UV λ_{max} (ϵ_{max}); pH 1, 291 nm (9.7 x 10^3), 253 (10.8 x 10^3); pH 7, 289 (9.5 x 10^3), 253 (10.6 x 10^3); pH 11, 266 (14.0 x 10^3); FAB/MS $(MH)^+$, obsd 298; (MH^+-NH),283; (BH^+), 166; (BH^+-NH), 151. Anal calcd for $C_{10}N_{16}N_7O_4Cl.H_2O$: C, 34.14; H, 5.15; N, 27.8. Found: 34.13; H, 4.89; N, 27.25.

1-Amino-6-thioguanosine (2b)

The same procedure was adopted for the preparation of **2b** as described previously for **1c**; compound **7** (2 g, 6 mmol) in H_2O (17 mL) was treated with freshly distilled pyridine (12.6 mL) followed by the addition of liquid H_2S (45 mL). The precipitate obtained after the work-up was recrystallized from H_2O and EtOH. Yield: 1.23 g (65%); mp 239-241°C; ^{1}H-NMR $(CD_3)_2SO$; δ8.17 (s, 1H, H8), 7.50 (s, 2H, 2-NH_2), 6.24 (s, 2H, 1-NH_2), 5.73 (d, 1H, H1′), 5.45 (d, 1H, OH), 5.16 (d, 1H, OH), 5.02 (bs, 1H, OH), 4.42 (d, 1H, H2′), 4.11 (d, 1H, H3′), 3.89 (d, 1H, H4′), 3.58 (m, 2H, H5′+H5") ppm; UV λ_{max} (ϵ_{max}); pH 1, 344 nm (21.0 x 10^3), 262 (10 x 10^3); pH 7, 337 (25.0 x 10^3), 255 (10.5 x 10^3); pH 11, 337 (25.2 x 10^3), 255 (10.5 x 10^3); FAB/MS $(MH)^+$ obsd 315, $C_{10}H_{15}N_6O_4S$ requires 315. Anal calcd for $C_{10}H_{14}N_6O_4S$: C, 38.21; H, 4.48; N, 26.73; S, 10.19. Found: C, 38.35; H, 4.54; N, 26.74; S, 10.38.

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