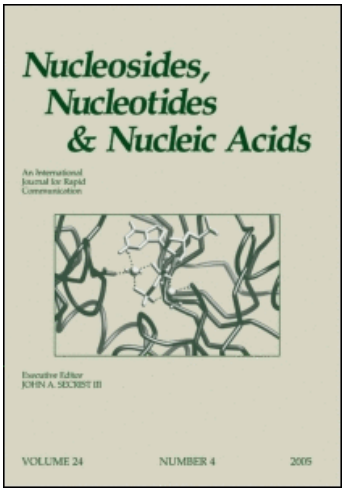

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NUCLEIC ACID RELATED COMPOUNDS. 82. CONVERSIONS OF ADENOSINE TO INOSINE 5'-THIOETHER DERIVATIVES WITH *ASPERGILLUS ORYZAE* ADENOSINE DEAMINASE OR ALKYL NITRITES. SUBSTRATE AND INHIBITORY ACTIVITIES OF INOSINE 5'-THIOETHER DERIVATIVES WITH PURINE NUCLEOSIDE PHOSPHORYLASE[§,1]

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Abstract: Adenosine derivatives lacking a 5'-hydroxyl group seldom act as alternative substrates of adenosine deaminases from calf intestine and other mammalian sources. A deaminase from *Aspergillus oryzae* deaminated adenosine 5'-thioether derivatives cleanly and more efficiently than alkyl nitrites. The inosine derivatives were very poor alternative substrates and weak inhibitors of purine nucleoside phosphorylase.

Introduction

The synthesis of inosine derivatives can be achieved by chemical transformations of inosine.[2,3] Protection of the 2',3'-diol unit, 5'-hydroxyl group activation, displacement with thiolate salts, and deprotection have been used for the preparation of 5'-*S*-methyl-5'-thioinosine (MTI),[3a] 5'-*S*-isobutyl-5'-thioinosine (SIBI)[3d] and *S*-inosyl-L-homocysteine (SIH, InoHcy).[3b,c] However, it often is more convenient and efficient to deaminate their adenosine analogues since the chemistry of adenosine is much better established. Chemical deaminations had been reported earlier,[4,5a] but these procedures suffer from incomplete reaction and by-product formation. Enzymatic deaminations[5,6] are clean and quantitative with effective alternative substrates, but deamination rates vary widely. Our earlier study with the adenosine deaminase (ADA) from calf intestine (adenosine aminohydrolase EC 3.5.4.4) demonstrated that numerous sugar-modified adenosine derivatives were converted

[§]This paper is dedicated to the late Professor Roland K. Robins.

into their inosine counterparts if an unsubstituted 5'-hydroxyl group was maintained in a reasonably unrestricted environment within the carbohydrate moiety.[6] Replacement of the 5'-OH of adenosine with a methylthio group to give 5'-*S*-methyl-5'-thioadenosine (MTA) resulted in loss of substrate activity.[5a,6] However, it was found[5b,c] that a "non-specific" adenosine deaminase from *Aspergillus oryzae* converted MTA, *S*-adenosylhomocysteine (SAH, AdoHcy), and their sulfoxides to inosine derivatives; but adenosine sulfonium compounds such as *S*-adenosylmethionine (SAM, AdoMet) were not deaminated.

The early demonstration of Levene and Jacobs[4a] that nitrous acid converted adenosine to inosine has been used extensively in nucleic acid chemistry.[2b] Nitrosyl chloride in DMF converted adenosine 1-*N*-oxide into inosine 1-*N*-oxide.[4b] Deaminations of 5'-modified adenosines to inosine analogues with nitrous acid have been reported.[4c,5a] Those reactions presumably proceed via unstable diazonium salts.[2b] Diazotization in aqueous acid was employed for the introduction of fluorine and chlorine at C2 of purine nucleosides.[7,8] Convenient nonaqueous diazotization/halodediazonization procedures with *tert*-butyl nitrite were developed for the introduction of fluoro, chloro, and bromo groups at C2 of purine nucleosides.[9] Incorporation of halogens at C6 of the purine ring occurred upon heating and photolysis of 9-substituted adenines in halogenated solvents containing pentyl nitrite.[10] These results were attributed to radical abstraction processes, and Nair and Richardson also synthesized the antibiotic nebularine[11] ("purine riboside") from adenosine under anhydrous diazotization/reductive deamination conditions.

We first reported 5'-*S*-alkyl(or aryl)-5'-fluoro-5'-thionucleosides[12] which hydrolyze to give mechanism-based inhibitors of *S*-adenosyl-L-homocysteine hydrolase.[13] This chemistry provided intermediates for the synthesis of other potent inhibitors of AdoHcy hydrolase,[13,14] and McCarthy and co-workers[14] have developed potent biologically active agents from fluorinated 5'-thionucleosides. 5'-Fluorinated MTA analogues have been evaluated as substrates and inhibitors of methylthioadenosine phosphorylase (MTAPase).[15] Other 5'-fluorinated MTA derivatives have been studied as inhibitors of MTAPase and cultured cells.[16]

MTA[17] and 5'-deoxy-5'-haloadenosine derivatives[18] undergo cleavage by MTAPase to give 5-*S*-methyl-5-thioribose-1-phosphate and 5-halo-5-deoxyribose-1-phosphate,

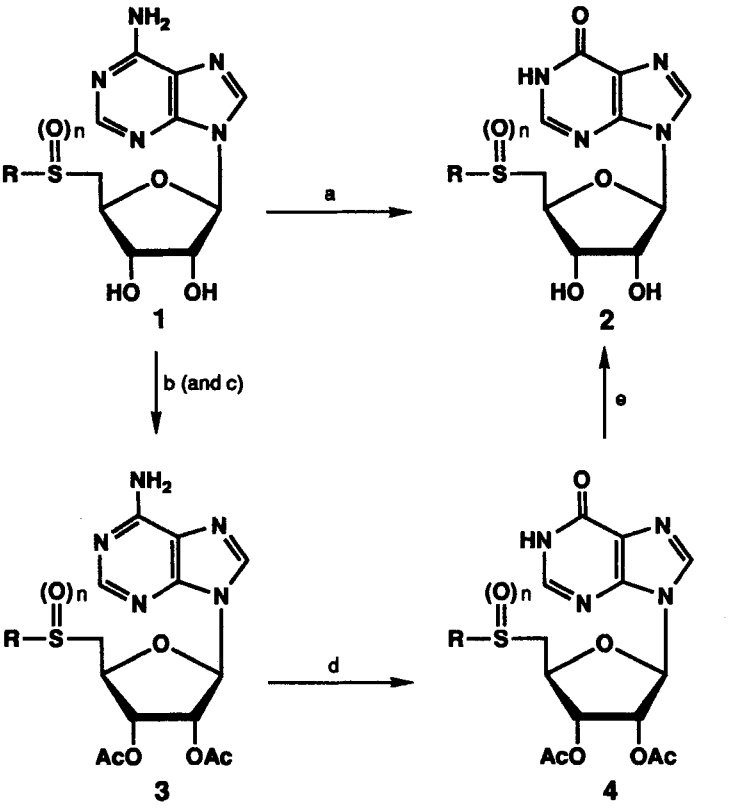
respectively. Analogous 5'-deoxy-5'-haloinosine derivatives produced parallel biological effects in cell lines deficient in MTPase but with normal levels of purine nucleoside phosphorylase (PNP), an enzyme which catalyzes rapid phosphorolysis of hypoxanthine, but not adenine, nucleosides.[18a] Stoeckler *et al.* have shown that replacement of the 5'-hydroxyl group of inosine by halogen or thioalkyl resulted in diminished alternative substrate activity for PNP.[4c] We now report two mild methods for transformations of 5'-thioadenosine precursors **1** to 5'-thioinosine derivatives **2** by enzymatic and chemical processes, and the inefficient alternative substrate and weak inhibitory activities of these inosine derivatives with human erythrocyte PNP.

Results and Discussion

As expected,[6] ADA from calf intestine failed to accept the 5'-thioadenosine derivatives **1** as alternative substrates. However, a Sigma Chemical Co. preparation of α-amylase from *Aspergillus oryzae* effected deamination of **1a-f** (pH 6.8 buffer containing ethanethiol) to give inosine compounds **2a-f**. The reaction usually was complete in 6-12 h for **1a-d**, but needed further time (10-18 h) for sulfoxide **1e**(S_R) and sulfone **1f**. Deamination progress was conveniently monitored by TLC (inosine products **2a-f** were more polar) or UV spectroscopy (hypsochromic shift, e.g. λ_{max} 259 → 246 nm for **1a** → **2a**). The 5'-thioinosine products **2a-f** were isolated in high yields (76-93%) after purification on a column of Amberlite XAD-4 resin followed by recrystallization.

Alkyl nitrites in tetrahydrofuran (THF) or ethyl acetate effected mild, nonaqueous conversions of 5'-substituted adenosine to inosine derivatives under neutral conditions. This has potential applicability to adenine nucleosides which are unstable in acid. Treatment of 2',3'-di-*O*-acetyl-5'-*S*-phenyl-5'-thioadenosine (**3c**) with pentyl nitrite in THF (4 h, reflux) gave inosine product **4c** (19%) plus the less polar 2',3'-di-*O*-acetyl-5'-*S*-phenyl-5'-thionebularine (38%). The nebularine derivative, presumably formed by diazotization/reductive deamination, had spectroscopic data analogous to those for similar purine nucleosides.[10b] Analogous treatment of **3a** with pentyl nitrite/THF gave the inosine product **4a** (18%) and 2,3-di-*O*-acetyl-5'-*S*-methyl-5'-thionebularine (24 %).

Treatment of **3c** with *tert*-butyl nitrite (TNB) in THF (8 h, ambient temperature) gave the nebularine (15%) plus inosine **4c** (29%) derivatives in a reversed ratio. These



(a) α-Amylase; (b) Ac_2O/pyridine; (c) MCPBA; (d) *tert*-Butyl nitrite/EtOAc; (e) NH_3/MeOH.

diazotization/hydroxydediazoniations with adenosine derivatives gave inosine analogues as the highly predominant products and in higher yields when ethyl acetate was used as solvent. Thus, treatment of **3a** and **3c** with TBN/EtOAc (ambient temperature, 5-9 h) gave 5'-thioinosine derivatives **2a** and **2c** in moderate yields after deprotection (NH_3/MeOH).

Adenosine 5'-derivatives with sulfur in higher oxidation states gave the inosine analogues in higher yields upon treatment with TBN/EtOAc. Thus, sulfones **3f** and **3g** (obtained by acetylation and oxidation of **1a** and **1c**, respectively) gave **4f** and **4g** in good yields with TBN/EtOAc (ambient temperature, 14 h). Deprotection gave **2f** and **2g**.

Table I. Substrate and Inhibitory Activities of 5'-Modified Inosines with Human Erythrocyte PNP[a]

Compound	Substrate Activity[b]	% Inhibition[c]
2a	0.01	61
2b	0.007	33
2c	0.004	38
2d	0.003	37
2e(S_R)	<0.001	46
[d]	<0.001	36
2f	<0.001	18
2g	0	38

[a]Results are means of two determinations. [b]Tested at 200 μM concentration; expressed as μmol cleaved/min/unit of PNP. [c]Phosphorolysis of 40 μM inosine in the presence of 100 μM analogue; values are expressed as percent inhibition of the control rate. [d]5'-Deoxy-5'-(phenylsulfinyl)inosine [prepared by TBN/EtOAc deamination of 2',3'-di-*O*-acetyl-5'-deoxy-5'-(phenylsulfinyl)adenosine[13b] and deprotection].

These hydroxydeamination reactions presumably occur via diazonium intermediates which undergo attack by water to produce the hypoxanthine nucleosides. It is noteworthy that one equivalent of water is released during diazotization. Addition of water or dilute aqueous base (NaOH) to the reaction mixtures neither improved the yields nor changed the ratios of inosine/nebularine products significantly.

The inosine analogues were examined as substrates and inhibitors of human erythrocyte PNP, and the results are presented in Table I. At 200 μM concentrations under the specified assay conditions, substrate activity was detected with all analogues except **2g**. Known 5'-*S*-methyl-5'-thioinosine (MTI, **2a**) and 5'-*S*-isobutyl-5'-thioinosine (SIBI, **2b**) standards were included for comparison, and underwent phosphorolysis at respective rates of 0.01 and 0.007 μmol/min/unit of PNP. In a previous study,[4c] these nucleosides produced K_m values of 15 and 42 μM; and V_{max} values of 0.7% and 0.2%, respectively, relative to inosine. In that study, *S*-benzyl and *S*-alkylcarboxylic acid derivatives also resisted enzyme-mediated phosphorolysis. Of the analogues presented here, all were

cleaved less rapidly than MTI (**2a**). In every case, the thioether derivatives were substantially more reactive than their analogues with sulfur in higher oxidation states.

The standards **2a** and **2b** caused approximately 61% and 33% inhibition, respectively, when tested as inhibitors of inosine phosphorolysis at a concentration (100 μM) that was 2.5-fold higher than that of the substrate. These compounds were previously found to have respective K_i values of 22 and 105 μM.[4c] It is noteworthy that **2g**, which was inert as a substrate, displayed inhibitory activity comparable to those of SIBI (**2b**) and most of the other analogues. Among these compounds, no apparent correlation exists between substrate activity and inhibitory potency. A possible rationalization of these observations can be derived from X-ray crystal structures of nucleosides complexed with human erythrocyte PNP.[19] Nucleosides in these complexes bind in a *syn* conformation with the 5'-hydroxyl group extending into the solvent. This strained conformation might be stabilized by a hydrogen-bond between the 5'-OH and a histidine sidechain which moves into place after a substrate-induced conformational change in the protein. Although bulky 5'-substituents might be accommodated, their substitution for the 5'-OH might perturb the conformation of the nucleoside substrate or its orientation in the protein. Thus, such 5'-modified analogues might bind with relatively high affinities but be poorly oriented for the catalytic phosphorolysis step.

Experimental Section

Uncorrected melting points were determined on a microstage block. UV spectra (MeOH) were recorded with a Hewlett Packard 8451A diode array spectrophotometer. ^{13}C (Table II) and ^{1}H (Table III) NMR spectra (Me_4Si/Me_2SO-d_6) were determined with a Varian Gemini 200 spectrometer at 50 and 200 MHz, respectively. Low resolution electron impact mass spectra (MS) at 20 eV were determined by direct probe sample introduction with a Finegan MAT 8430 spectrometer. Elemental analyses were determined by the microanalytical laboratories at Adam Mickiewicz University, Poznan, Poland or M-H-W laboratories, Phoenix, AZ. Commercial reagents and solvents of reagent quality were purified and dried. Pentyl nitrite and *tert*-butyl nitrite (TBN) were prepared essentially as described.[9,20,21] TBN was distilled at -5 °C into a cold trap at -78 °C with water aspirator vacuum. An "α-amylase" preparation from *Aspergillus oryzae* (Crude; Type X-A) and adenosine deaminase (adenosine aminohydrolase EC 3.5.4.4) from calf intestinal mucosa

Table II. ^{13}C NMR Spectral Data[a,b]

Comp'd.	C2	C4	C5	C6	C8	C1'	C2'[c]	C3'[c]	C4'	C5'
2a[d]	146.29	148.74	124.77	156.94	139.32	87.52	73.23	72.66	84.07	36. 04
2b[e]	146.19	148.73	124.64	156.92	139.46	87.52	73.00	72.43	84.39	34. 46
2c[f]	146.14	148.73	124.69	156.90	139.45	87.49	73.05	72.52	83.15	35. 09
2d[g]	146.12	148.75	124.72	156.90	139.49	87.42	72.99	72.46	83.35	37. 37
2e(S_R)[h]	146.13	148.57	124.92	156.90	139.90	88.04	73.03	72.97	78.59	60. 66
2f[i]	146.26	148.51	124.07	156.92	139.68	88.15	72.87	72.77	78.99	56. 89
2g[j]	145.94	148.27	124.83	156.81	139.82[k]	87.93	73.15	72.24	79.32	58. 13

[a]Chemical shifts (δ ppm) at 50 MHz. [b]All proton-decoupled peaks appeared as singlets. [c]Assignments might be reversed. [d]Peak also at δ 15.57 (CH_3S). [e]Peaks also at δ 21.57, 21.63 (CH_3), 27.98 (CH_2), 41.13 (CH). [f]Peaks also at δ 126.21, 128.53, 129.38, 135.91 (Ar). [g]Peaks also at δ 55.27 (CH_3O), 114.99, 125.64, 132.70, 158.85 (Ar). [h]Peaks also at δ 55.60 (CH_3O), 115.11, 126.28, 135.39, 161.92 (Ar). [i]Peak also at δ 42.12 (CH_3SO_2). [j]Peaks also at δ 128.00, 129.10, 133.85, 139.82 (overlapped with C8 signal). [k]Overlapped with C1" phenyl signal.

(Type II) were purchased from Sigma Chemical Co. Thin layer chromatography (TLC) was performed on Merck Kieselgel 60 F_{254} sheets (detection under 254 nm light) with the following solvents: S_1 (EtOAc/*i*-PrOH/H_2O; 4:2:1, upper layer), S_2 ($CHCl_3$/MeOH; 5:1) and S_3 ($CHCl_3$/MeOH: 9:1). Amberlite XAD-4 nonionic polymeric absorbent (20-60 mesh polystyrene resin from Aldrich) or Merck kieselgel 60 (230-400 mesh) were used for column chromatography. The 5'-substituted adenosine starting materials were prepared as described: **1a**,[22] **1b**,[23] **1c,d**,[22] **1e**(S_R),[24] and **1f**.[25] Acetylations (Ac_2O/pyridine) of **1a** and **1c** to give **3a** and **3c** occurred quantitatively.[12,13b]

Deamination of 5'-substituted adenosines 1a-f with "α-amylase". *General Procedure for 2a-f.* Samples of **1a-f** (0.25 mmol) were dissolved in H_2O (50 mL) and 0.1 M phosphate buffer (pH 6.8, 100 mL), and in some cases heating was necessary. Crude α-amylase (~25 mg) and ethanethiol (74 μL, 62 mg, 1 mmol) were added and stirring at

Table III. ^{1}H NMR Spectral Data[a,b]

Compound	H1[c] ($J_{1'-2'}$)	H2[d] ($J_{2'-3'}$)	H3[d] ($J_{3'-4'}$)	H4[d] ($J_{4'-5'}$)	H5[e] ($J_{5'-5''}$)	H5''[e,f] ($J_{5''-4'}$)	H2[g]	H8[g]	NH[h]	OH2[c] (^3J)	OH3[c] (^3J)	Aromatic (J_{A-B}) and/or others
2a	5.87 (5.8)	4.62 (5.0)	4.09 (4.0)	4.02 (5.8)	2.86 (14.0)	2.75 (6.8)	8.08	8.35	12.40	5.53 (5.8)	5.34 (4.4)	2.04[g](CH_3S)
2b	5.86 (5.8)	4.64 (5.1)	4.10 (3.8)	4.01 (5.7)	2.87 (14.0)	2.76 (6.8)	8.06	8.36	12.40	5.54 (6.2)	5.33 (4.8)	0.83,[c] 0.85[c] (6.6[i], 2 x CH_3) 1.66[j](6.8[k], CH), 2.35[c](6.9[l], CH_2)
2c	5.87 (6.0)	4.68[e] (4.8)	4.15[e] (3.7)	4.00 (5.9)	3.40 (13.8)	3.27 (7.0)	8.06	8.37	12.40	5.57[h]	5.41[h]	7.18-7.40[m]
2d	5.85 (6.1)	4.68[e] (5.2)	4.11[e] (3.3)	3.93 (6.2)	3.26 (13.9)	3.13 (7.0)	8.07	8.36	12.38	5.55[h]	5.38[h]	6.89[c], 7.38[c](8.5) 3.73[g](OCH_3)
2e(S_R)	5.94 (5.7)	4.67 (4.8)	4.13 (3.5)	4.30 (10.3)	3.31 (13.0)	3.10 (3.1)	8.05	8.38	12.40	5.62 (6.0)	5.49 (4.8)	7.11[c], 7.61[c](8.5) 3.80[g](OCH_3)
2f	5.91 (5.6)	4.60 (4.9)	4.16 (3.5)	4.30 (9.5)	3.83 (15.5)	3.45 (2.6)	8.06	8.38	12.40	5.66 (6.0)	5.57 (5.0)	2.85[g](CH_3SO_2)
2g	5.76 (6.3)	4.68 (4.8)	4.10 (3.0)	4.25 (8.5)	4.02 (14.4)	3.76 (3.3)	8.02	8.18	12.39	5.57 (6.2)	5.54 (4.7)	7.41-7.80[m]
4f	6.24 (5.7)	5.93[e] (5.5)	5.58[e] (4.3)	4.57 (8.2)	3.97 (15.0)	3.69 (3.0)	8.13	8.39	12.40			2.01,[g] 2.12[g] (Ac's) 2.90[g] (CH_3SO_2)
4g	6.09 (6.4)	6.03[e] (5.0)	5.52[e] (3.4)	4.55 (8.7)	4.22 (15.0)	3.95 (3.3)	8.03	8.12	12.40			1.95,[g] 2.10[g] (Ac's) 7.35-7.80[m]

[a]Chemical shifts (δ) at 200 MHz in Me_4Si/Me_2SO-d_6. [b]"Apparent" first order coupling constant (in parentheses). [c]Doublet (unless otherwise noted). [d]Doublet of doublets of doublets (unless otherwise noted). [e]Doublet of doublets (unless otherwise noted). [f]Upfield resonance assigned to 5" (pro-*R*)-H. [g]Singlet. [h]Broad singlet. [i]($^3J_{(CH_3)_2CH}$). [j]Septet. [k]($^3J_{(CH_3)_2CH\text{-}CH_2}$). [l]($^3J_{CH_2\text{-}CH}$). [m]Multiplet.

ambient temperature was continued until TLC (S_1 or S_2) indicated complete conversion of **1** to **2** (normally 6-18 h; more time is required for the deaminations of **1e** and **f**). After concentration of the mixtures to ~10 mL at <30 °C, the residues were purified on a column of XAD-4 resin (1.5 x 25 cm; washed with Me_2CO, MeOH/H_2O, and H_2O before use). Elution was effected with H_2O (300 mL) followed by MeOH/H_2O (1:1, 200 mL) and MeOH (400 mL). Evaporation of the appropriate fractions (usually only MeOH fractions with inosine-like UV absorption) gave the crude inosine derivatives **2a-f**.

2a: 69 mg (93%, from MeOH/H_2O); mp 217-219 °C (lit.[3a] mp 220-221 °C); UV max 246 nm (ε 11 100), min 223 nm (ε 3500); MS *m/z* 298 (1, M^+), 195 (28), 137 (48, BH_2), 136 (100, BH).

2b: 70 mg (82%, "diffusion crystallization"[26] MeOH/EtOAc//hexanes); mp 142-147 °C (dec) (lit.[3d] mp 179-181 °C); UV max 246 nm (ε 10 900), min 222 (ε 3900); MS *m/z* 340 (1, M^+), 205 (48), 137 (24, BH_2), 136 (48, BH), 103 (100). Anal. Calcd for $C_{14}H_{20}N_4O_4S$ (340.4): C, 49.40; H, 5.92; N, 16.46. Found: C, 49.20; H, 5.87; N, 16.29.

2c: 70 mg (78%, from MeOH/H_2O); mp 182-183 °C; UV max 250 nm (ε 18 500), min 226 (ε 7700); MS *m/z* 360 (1, M^+), 224 (78), 137 (33, BH_2), 136 (100, BH), 123 (90), 110 (95). Anal. Calcd for $C_{16}H_{16}N_4O_4S$ (360.4): C, 53.32; H, 4.48; N, 15.55. Found: C, 53.58; H, 4.62; N, 15.53.

2d: 81 mg (83%, from MeOH/H_2O); mp 171-173 °C; UV max 252, 232 nm (ε 19 500, 17 200), min 238, 220 nm (ε 16 700, 14 200); MS *m/z* 390 (1, M^+), 301 (36), 283 (92), 254 (21), 171 (100), 140 (28), 136 (34, BH), 112 (88). Anal. Calcd. for $C_{17}H_{18}N_4O_5S$ (390.4): C, 52.30; H, 4.65; N, 14.35. Found: C, 52.15; H, 4.80; N, 14.49.

2e(S_R): 92 mg (91%, from MeOH/H_2O); mp 150-155 °C; UV max 246 nm (ε 20 500), min 220 (ε 7500); MS *m/z* 406 (1, M^+), 310 (10), 278 (100), 156 (24), 155 (25), 139 (91). Anal. Calcd. for $C_{17}H_{18}N_4O_6S$ (406.4): C, 50.24; H, 4.46; N, 13.79. Found: C, 49.95; H, 4.72; N, 13.60.

2f: 63 mg (76%, "diffusion crystallization"[26] MeOH/EtOAc); mp 151-153 °C; UV max 246 nm (ε 10 500), 222 (ε 3400); MS *m/z* 330 (2, M^+), 137 (3, BH_2), 136 (100, BH), 80(11). Anal. Calcd. for $C_{11}H_{14}N_4O_6S$ (330.3): C, 40.00; H, 4.27; N, 16.96. Found: C, 40.02; H, 4.47; N, 16.83.

2',3'-Di-*O*-acetyl-5'-deoxy-5'-(phenylsulfonyl)adenosine (3g). MCPBA (624 mg of 85% reagent, 3.08 mmol) in CH_2Cl_2 (30 mL) was added dropwise to a stirred solution of **3c**[13b] (619 mg, 1.4 mmol) in CH_2Cl_2 (30 mL) at -20 °C. After 3 h the temperature was allowed to slowly rise to ambient (~1 h) and stirring was continued for ~15 min. Saturated $NaHCO_3/H_2O$ was added, stirring was continued for 10 min, and the layers were separated. The aqueous layer was extracted ($CHCl_3$) and the combined organic phase was washed (H_2O and brine), dried ($MgSO_4$), and evaporated. The residue was column chromatographed (MeOH/$CHCl_3$, 1:49) to give **3g** (570 mg, 86%) as a white foam: ^{1}H NMR δ 1.94 (s, 3, Ac), 2.11 (s, 3, Ac), 3.96 (dd, $J_{5''-5'}$ = 15.0 Hz, $J_{5''-4'}$ = 3.1 Hz, 1, H5''), 4.33 (dd, $J_{5'-4'}$ = 9.7 Hz, 1, H5'), 4.53 (ddd, $J_{4'-3'}$ = 2.8 Hz, 1, H4'), 5.57 (dd, $J_{3'-2'}$ = 5.0 Hz, 1, H3'), 6.11 (d, $J_{1'-2'}$ = 6.4 Hz, 1, H1'), 6.20 (dd, 1, H2'), 7.30-7.71 (m, 5, Ph), 8.12 (s, 1, H2), 8.14 (s, 1, H8); MS *m/z* 475 (18, M^+), 139 (100).

2',3'-Di-*O*-acetyl-5'-deoxy-5'-(methylsulfonyl)adenosine (3f). Oxidation of **3a**[13b] (533 mg, 1.4 mmol) with MCPBA (624 mg, of 85% reagent, 3.08 mmol) (as described above for **3g**) gave **3f** (460 mg, 80%) as a white foam: ^{1}H NMR δ 2.00 (s, 3, Ac), 2.12 (s, 3, Ac), 2.85 (s, 3, CH_3SO_2), 3.70 (dd, $J_{5''-5'}$ = 9.2 Hz, $J_{5''-4'}$ = 2.5 Hz, 1, H5''), 4.03 (dd, $J_{5'-4'}$ = 9.2 Hz, 1, H5'), 4.55 (ddd, $J_{4'-3'}$ = 4.3 Hz, 1, H4'), 5.66 (dd, $J_{3'-2'}$ = 5.4 Hz, 1, H3'), 6.06 (dd, $J_{2'-1'}$ = 5.8 Hz, 1, H2'), 6.26 (d, 1, H1'), 8.20 (s, 1, H2), 8.41 (s, 1, H8); MS *m/z* 413 (35, M^+), 279 (66), 139 (100).

5'-Deoxy-5'-(phenylsulfonyl)inosine (2g). (a) *Deamination with* tert-*butyl nitrite/ethyl acetate.* TBN (0.5 mL) was added dropwise (syringe) to a stirred solution of **3g** (250 mg, 0.5 mmol) in anhydrous EtOAc (50 mL) at ambient temperature. Stirring was continued (~16 h) until TLC (S_1 or S_3) showed conversion of **3g** to the more polar **4g**. The mixture was evaporated, partitioned ($NaHCO_3/H_2O//CHCl_3$), and the organic layer was washed (brine), dried ($MgSO_4$), and evaporated. Column chromatography of the residue (MeOH/$CHCl_3$, 3:97) gave **4g** (175 mg, 73%): mp 229-231 °C (from MeOH); UV max 248 nm (ε 13 600), min 229 nm (ε 4900); MS *m/z* 476 (21, M^+), 319 (56), 136 (100, BH). Anal. Calcd. for $C_{20}H_{20}N_4O_8S$ (476.4): C, 50.42; H, 4.23; N, 11.76. Found C, 50.05; H, 4.34; N, 12.09. (b) *Deprotection.* A solution of **4g** (100 mg, 0.21 mmol) in MeOH (10 mL) was stirred with saturated NH_3/MeOH (10 mL) at ~0 °C (ice bath) for 2 h and evaporated. The creamy solid was recrystallized (2 x MeOH) to give **2g**

(58 mg, 70%) as colorless crystals: mp 225-230 °C (dec); UV max 249 nm (ε 13 300), min 230 nm (ε 6100); MS *m/z* 392 (2, M^+), 142 (22), 136 (58, BH), 78 (100). Anal. Calcd. for $C_{16}H_{16}N_4O_6S$ (392.39): C, 48.98; H, 4.11; N, 14.28. Found: C, 48.72; H, 3.94; N, 14.07.

5'-Deoxy-5'-(methylsulfonyl)inosine (2f). Treatment of **3f** (200 mg, 0.48 mmol) with TBN (0.5 mL) and deprotection of crude **4f** [MS *m/z* 414 (22, M^+), 319 (52), 100 (100)] [(as described for **2g**, steps (a) and (b)] followed by silica column chromatography (EtOAc → S_1) and crystallization (MeOH) gave **2f** (87 mg, 55%) with data identical to this compound obtained by enzymatic deamination.

5'-*S*-Phenyl-5'-thioinosine (2c). (a) *Deamination with TBN/EtOAc.* Treatment of **3c**[13b] (100 mg, 0.22 mmol) in EtOAc (50 mL) with TBN (0.8 mL) [as described for **3g**, step (a)] for 9 h at ambient temperature gave **4c** (55 mg, 55%): UV max 252 nm; ^{1}H NMR δ 1.98 (s, 3, Ac), 2.09 (s, 3, Ac), 3.37-3.58 (m, 2, H5',5"), 4.20-4.28 (m, 1, H4'), 5.55 (dd, $J_{3'-4'}$ = 4.0 Hz, $J_{3'-2'}$ = 5.9 Hz, 1, H3'), 6.00 (dd, $J_{2'-1'}$ = 5.8 Hz, 1, H2'), 6.17 (d, 1, H1'), 7.20-7.41 (m, 5, Ph), 8.10 (s, 1, H2), 8.33 (s, 1, H8), 12.41 (br s, 1, NH); MS *m/z* 444 (8, M^+), 201 (29), 139 (100), 97 (96). (b) *Deprotection.* Treatment of **4c** (44 mg, 0.1 mmol) with NH_3/MeOH [as described for **2g**, step (b)] and purification on a XAD-4 column (as described in the general enzymatic deamination procedure) gave **2c** (22 mg, 61%; data identical to **2c** obtained by enzymatic deamination).

(b) *Deamination with TBN/THF.* Treatment of **3c** (110 mg, 0.25 mmol) with TBN (0.4 mL) in THF (4 mL) at ambient temperature (8 h) and silica column chromatography (MeOH/$CHCl_3$, 1:49 → 1:19) gave in order of elution: 2',3'-di-*O*-acetyl-5'-*S*-phenyl-5'-thionebularine [(15 mg, 15%): UV max 256 nm; ^{1}H NMR δ 1.99 (s, 3, Ac), 2.11 (s, 3, Ac), 3.40-3.60 (m, 2, H5',5"), 4.25-4.33 (m, 1, H4'), 5.65 (dd, $J_{4'-3'}$ = 4.3 Hz, $J_{3'-2'}$ = 5.4 Hz, 1, H3'), 6.16 (dd, $J_{2'-1'}$ = 5.9 Hz, 1, H2'), 6.34 (d, 1, H1'), 7.18-7.36 (m, 5, Ph), 8.80 (s, 1, H2), 8.99 (s, 1, H8), 9.23 (s, 1, H6); MS *m/z* 428 (8, M^+), 368 (8), 165 (36), 139 (100), 97 (94)], unchanged **3c** (12 mg, 11%), and **4c** (32 mg, 29%).

(c) *Deamination with pentyl nitrite/THF.* Analogous treatment of **3c** with pentyl nitrite (0.5 mL) in THF (4 mL) (ambient temperature, 4 h; reflux, 1 h) gave 2',3'-di-*O*-acetyl-5'-*S*-phenyl-5'-thionebularine (38 mg, 38%) and **4c** (21 mg, 19%).

The addition of H_2O (0.1 mL) or 1N NaOH (0.1 mL) had little effect on the ratios or yields of products.

5'-*S*-Methyl-5'-thioinosine (2a). (a) *Deamination with TBN/EtOAc.* Treatment of **3a**[13b] (100 mg, 0.26 mmol) in EtOAc (50 mL) with TBN (0.8 mL) [5 h, as described for **2g**, step (a)] gave **4a** (33 mg, 33%): UV max 248 nm; ^{1}H NMR ($CDCl_3$) δ 2.02 (s, 3, Ac), 2.12 (s, 3, Ac), 2.14 (s, 3, CH_3S), 2.89 (dd, $J_{5''-5'}$ = 14.5 Hz, $J_{5''-4'}$ = 5.6 Hz, 1, H5''), 2.93 (dd, $J_{5'-4'}$ = 5.0 Hz, 1, H5'), 4.40 (ddd, $J_{4'-3'}$ = 4.6 Hz, 1, H4'), 5.55 (dd, $J_{3'-2'}$ = 5.6 Hz, 1, H3'), 5.89 (dd, $J_{2'-1'}$ = 5.5 Hz, 1, H2'), 6.11 (d, 1, H1'), 8.02 (s, 1, H2), 8.18 (s, 1, H8), 10.53 (br s, 1, NH); MS *m/z* 382 (35, M^+), 368 (6), 284 (100), 256 (59). TLC (S_1, S_3) showed **4a** as the sole product. (b) *Deprotection.* Treatment of **4a** (25 mg, 0.065 mmol) with NH_3/MeOH [as described for **2g**, step (b)] gave **2a** (13 mg, 66%, from MeOH) with data identical to **2a** obtained by enzymatic deamination.

Analogous treatment of **3a** with pentyl nitrite (0.8 mL) in THF (4 mL) (2.5 h, reflux) gave **4c** (18 mg, 18%) and 2',3'-di-*O*-acetyl-5'-*S*-methyl-5'-thionebularine (23 mg, 24%): UV max 264 nm; ^{1}H NMR ($CDCl_3$) δ 2.01 (s, 3, Ac), 2.11 (s, 3, Ac), 2.12 (s, 3, CH_3S), 2.91 (dd, $J_{5''-5'}$ = 14.5 Hz, $J_{5''-4'}$ = 5.6 Hz, 1, H5''), 3.00 (dd, $J_{5'-4'}$ = 4.7 Hz, 1, H5'), 4.42 (ddd, $J_{4'-3'}$ = 4.5 Hz, 1, H4'), 5.56 (dd, $J_{3'-2'}$ = 5.7 Hz, 1, H3'), 5.99 (dd, $J_{2'-1'}$ = 5.5 Hz, 1, H2'), 6.21 (d, 1, H1'), 8.31 (s, 1, H2), 8.98 (s, 1, H8), 9.14 (s, 1, H6); MS *m/z* 366 (7, M^+), 306 (20), 263 (35), 259 (16), 145 (32), 139 (100), 121 (76).

Purine nucleoside phosphorylase (PNP) activity. The coupled spectrophotometric assays employed xanthine oxidase to convert the product hypoxanthine to uric acid, which absorbs at 293 nm.[27,28] Human erythrocyte PNP from Sigma was used without further purification. One unit of PNP activity is defined as the amount of enzyme which cleaves 1 μmol of inosine per min at 30 °C under standard conditions (500 μM inosine, 50 mM potassium phosphate, pH 7.4). Alternative substrate activity with 200 μM concentrations of the inosine analogues was tested in a 1 mL reaction volume containing 0.045 - 0.1 unit of PNP, 50 mM potassium phosphate, pH 7.4, and 0.04 unit of xanthine oxidase (Grade III, Sigma). The analogues were preincubated with all other reactants for 3-5 min prior to the addition of PNP.

Inhibition of inosine phosphorolysis. The inosine analogues (at 100 μM) were incubated in reaction mixtures which contained 0.007 unit of PNP and inosine at its K_m concentration (40 μM). Each analogue was preincubated for 3-5 min with the enzyme before addition of inosine. Substrate activities of the inosine analogues were negligible at this low concentration of PNP.

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