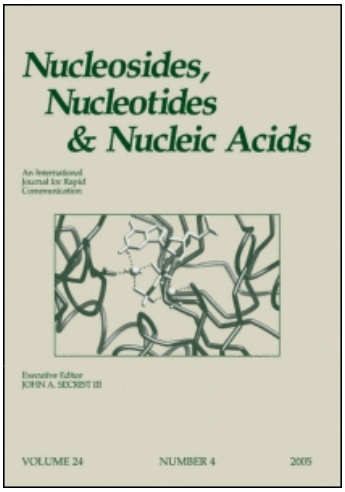


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

5'-Halogenated Formycins as Inhibitors of 5'-Deoxy-5'-methylthioadenosine Phosphorylase: Protection of Cells Against the Growth-Inhibitory Activity of 5'-Halogenated Adenosines

Shih Hsi Chu[a]; Lynn Ho[a]; Edward Chu[a]; Todd Savarese[a]; Zhi Hao Chen[a]; Elizabeth Claflin Rowe[a]; Ming Yu Wang Chu[a]
[a] Division of Biology and Medicine, Brown University, Providence, RI

To cite this Article Chu, Shih Hsi , Ho, Lynn , Chu, Edward , Savarese, Todd , Chen, Zhi Hao , Rowe, Elizabeth Claflin and Chu, Ming Yu Wang(1986) '5'-Halogenated Formycins as Inhibitors of 5'-Deoxy-5'-methylthioadenosine Phosphorylase: Protection of Cells Against the Growth-Inhibitory Activity of 5'-Halogenated Adenosines', Nucleosides, Nucleotides and Nucleic Acids, 5: 2, 185 — 200
To link to this Article: DOI: 10.1080/07328318608068672
URL: http://dx.doi.org/10.1080/07328318608068672

NUCLEOSIDES & NUCLEOTIDES, 5(2), 185-200 (1986)

5'-HALOGENATED FORMYCINS AS INHIBITORS OF 5'-DEOXY-5'-METHYLTHIOADENOSINE PHOSPHORYLASE: PROTECTION OF CELLS AGAINST THE GROWTH-INHIBITORY ACTIVITY OF 5'-HALOGENATED ADENOSINES

Shih Hsi Chu*, Lynn Ho, Edward Chu, Todd Savarese, Zhi Hao Chen, Elizabeth Claflin Rowe and Ming Yu Wang Chu

Division of Biology and Medicine
Brown University, Providence, RI 02912

Abstract: A series of 5'-halogenated formycins, including the chloro-, bromo- and iodo- derivatives, were synthesized. These compounds are competitive inhibitors of 5'-deoxy-5'-methylthioadenosine phosphorylase (MTAPase) with K_i values in the range of 10^{-7} M, making them the most potent inhibitors of MTAPase reported to date. These compounds protect cells from the growth-inhibitory action of 5'-halogenated adenosines, which must be activated by MTAPase. The syntheses of 5'-halogenated formycin B derivatives, which inhibit purine nucleoside phosphorylase, are also described.

Recent findings in the area of purine metabolism have suggested that inhibitors of 5'-deoxy-5'-methylthioadenosine phosphorylase (MTAPase) might be of use in chemotherapy. MTAPase catalyzes the reversible phosphorolytic cleavage of 5'-deoxy-5'-methylthioadenosine (MTA), which is produced from S-adenosyl-L-methionine during the synthesis of the polyamines, spermidine and spermine[1,2,3]:

MTA + P_i <====> Adenine + 5-Methylthio-α-D-ribose-1-phosphate

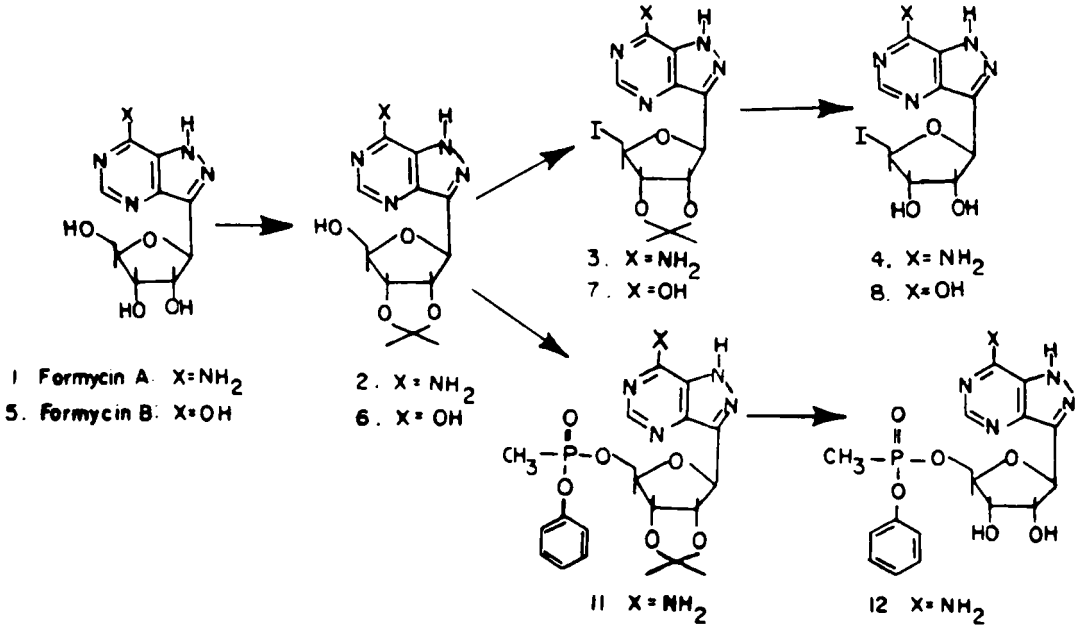
5-Methylthioribose-1-phosphate is converted to methionine[4-9] via a pathway which is not yet fully elucidated, and adenine is salvaged via adenine phosphoribosyltransferase (APRT) to AMP, thereby rejoining the adenine nucleotide pool[10,11]. MTAPase also functions

 0732-8311/86/0502-0185$3.50/0

to keep the intracellular concentration of MTA in the low micromolar range (<10 μM); MTA in the high micromolar range (100 μM or greater) has been demonstrated to be growth inhibitory to a number of cell systems (for a review, see ref. 12).

An MTAPase inhibitor may thereby produce a variety of metabolic inhibitions. For example, the salvage of adenine from MTA would be compromised in cells treated with such an inhibitor, forcing cells to become more dependent upon purine de novo synthesis. This would render cells more susceptible to growth inhibition by purine de novo synthesis inhibitors, e.g. methotrexate. In addition, MTA inhibitors would limit the salvage of the 5'-methythio group of MTA back to methionine, obligating cells to become more dependent upon exogeneous methionine. Finally, an inhibitor of MTAPase might elevate intracellular MTA levels, which, in turn, can cause a number of adverse effects, including 1) alterations of 3',5'-cyclic AMP metabolism[13,14], 2) inhibition of spermine and spermidine synthesis via product inhibition of spermine and spermidine synthase, respectively[15,16], and 3) inhibition of S-adenosylhomocysteine hydrolase[17,18]. The latter enzyme has the important function of cleaving S-adenosylhomocysteine, the product of AdoMet-dependent methylation reactions, to adenosine and homocysteine; without this enzyme, SAH could accumulate and inhibit critical methylations of macromolecules.

In designing an inhibitor of MTAPase, it was reasoned that the formycin analog of MTA might bind to the enzyme but, by virtue of its C-C glycosidic linkage, be resistant to phosphorolytic cleavage. By analogy, purine nucleoside phosphorylase (PNP, E.C.2.4.2.1), which is specific for 6-oxy purine nucleosides, is inhibited by the formycin analog of inosine, formycin B[19]. Although 5'-deoxy-5'-methylthioformycin A proved to be an inhibitor of MTAPase from murine Sarcoma 180 cells, an intermediate in its synthesis, 5'-chloro-5'-deoxyformycin A was several fold more potent. This prompted the synthesis of other 5'-halogenated formycins, which were also found to act as potent inhibitors of Sarcoma 180 MTAPase. Because of the 5'-halogenated formycins were found to be highly active against MTAPase, 5'-halogenated formycin B derivatives were also synthesized. These compounds have been shown by our colleagues to act as inhibitors of PNP[20]. In this report, the synthesis of 5'-halogenated formycin A and formycin B derivatives are described, and evidence is presented demonstrating that these compounds inhibit MTAPase and PNP, respectively, in intact cells.



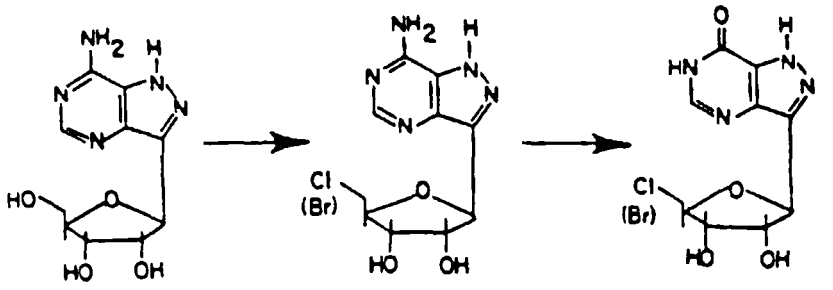
SCHEME I

RESULTS

Chemical Synthesis

The 2',3'-isopropylidene derivatives **2** and **6** were prepared in good yield by reaction of the formycin nucleosides **1** and **5** with acetone catalyzed by triethylorthoformate and p-toluenesulfonic acid.

Compound **6** was iodinated smoothly by methyltriphosphonium iodide at -70°C to the expected 5'-derivative **7** in 67% yield. However, compound **2** under the identical conditions yielded less than 5% of the desired product, **3**. Increasing the temperature to 0°C and adding the iodinating reagent as a slurry made it possible to isolate the product as a crystalline solid in 10-20% yields. Two major products of this reaction at either temperature resemble the anhydronucleosides obtained by J.Zemlicka[21] by heating either formycin with the bis-neopentyl acetal of dimethylformamide. Another by-product contained phosphorus and was identified as the 5'-phenoxymethylphosphonate ester **11** by deblocking to the 2',3'-dihydroxy ester **12**, characterized by NMR and elemental analysis.



1. 9. 13. 10. 14.

SCHEME II

Compounds 3 and 7 were deblocked by treatment with 90% formic acid, to yield 4 and 8 respectively. Because of the small amount of 3 available and the tendency of the 5'-iodo compound to form the presumed anhydronucleosides under basic conditions the product was not neutralized but collected as the formic acid salt.

Chlorination of 1 with thionyl chloride in DMF proceeded smoothly to give the 5'-chloroformycin A derivative 9, and sub-sequent deamination at C-7 with sodium nitrite in acetic acid gave the corresponding 5'-chloroformycin B, 10 in 61% yield.

Bromination of 1 with thionyl bromide using the same procedure yielded 5'-bromoformycin A, which on deamination with sodium nitrite in acetic acid gave a mixture of products which could be separated chromatographically. However, a preferable synthesis of 5'-bromoformycin B was by the method of Kashem[22], using triphenylphosphine and carbon tetrabromide. 5'-Bromoformycin B could also be prepared from the tosylate of formycin B with sodium bromide. Yields were about 30% in each case.

EXPERIMENTAL

Chemical Synthesis

Solvent removal was performed in vacuo at less than 35°C unless otherwise indicated. Melting points were determined on a Gallenkamp

apparatus and are uncorrected. UV spectra were taken on a Perkin-Elmer Model 402 spectrophotometer, and IR spectra obtained using a Perkin-Elmer Model 457 infra-red spectrophotometer. NMR spectra were recorded on a Varian A-60A, or Bruker 60-MHz spectrometer with TMS as an internal standard. NMR spectra for compounds **1**, **3**, **4** and **10** were subsequently checked using a Bruker WM-250 instrument. Assignment of the ribose proton resonances was made on the basis of a decoupling study of chloroformycin A between 3.8 and 5.2 ppm, using a Bruker WM-250 instrument. Coupling constants were determined for this compound and are shown under the synthesis of compound **10**. All compounds showed a similar pattern for ribose protons, with the exception of the anhydro by-products and phosphate esters (**11** and **12**). The protons of compounds for which coupling constants could not be accurately determined were assigned by analogy with respect to shape, approximate chemical shift and multiplicity of the 5 groups of peaks between 3.5 and 5.2, which in all cases were essentially identical in pattern to compound **10** but are reported as multiplets. In general, the pattern consisted of a doublet at 5.0-5.2 (C_1'-H), two triplets or pairs of doublets at 4.5-4.9 and 4.2 (C_2'-H and C_3'-H), a doubled triplet at 3.9-4.1 (C_4'-H) and a symmetrical 8-part multiplet (ddd) in the range 3.3-3.9 (2H, C_5'-H), frequently obscured in a broad hydroxyl or water peak centered at 3.3. The two C_5' protons are not equivalent due to asymetry at C_4'.

TLC was run on Merck silica gel 60 F-254 analytical plates visualized by UV. Preparative TLC plates were prepared using Merck PF-254 silica gel. Solvent systems were: (A) CH_2Cl_2-EtOH, 5:1, and (B) CH_2Cl_2-EtOH, 15:4. Elemental analyses were performed by the Galbraith Laboratories, Knoxville, TN, and the Baron Consulting Co, Orange, CT.

7-Amino-3-(2',3'-di-O-isopropylidine-β-D-ribofuranosyl)-pyrazolo-(3,4-d)-pyrimidine (2).

The isopropylidine derivative of formycin A was prepared according to the procedure of Tomasz[23]. 2.0 g (7.4 mmol) of dry **1** suspended in 85 ml of dry acetone containing 1.6 g of p-toluene sulfonic acid and 5.2 ml of triethylorthoformate was stirred overnight at room temperature, concentrated to 40 ml, neutralized to pH 8 with NH_4OH and evaporated. The residue, purified by flash chromatography

(CH_2Cl_2-MeOH, 5:2), yielded 2.4 g of 2 as an amorphous pale yellow solid. An analytical sample obtained from preparative TLC (solvent system B) melted at 102.7°; R_f(A), 0.27; UV(EtOH): λ_{max} 293 (9400); NMR(acetone-d_6): δ 1.22, 1.32, 1.58 (3s, 6H, CH_3 of ketal), 3.50-5.30 (4m and v.br.s overlap, 7H, CH of ribose-H and 5'-OH), 8.15 (s, 1H, C_5-H).

Anal. Calc'd for $C_{13}H_{17}N_5O_4$: C, 50.81; H, 5.58; N, 22.79. Found: C, 50.92, H, 5.93; N, 22.48.

7-Amino-3-(5'-iodo-5'-deoxy-2',3'-di-O-isopropylidene-β-D-ribofuranosyl)-pyrazolo-(3,4-d)-pyrimidine (3).

For the preparation of compound 3, modifications of the procedure of Verheyden and Moffatt[24] were studied under a variety of conditions in an attempt to raise the yield above 10-20%. The blocked iodoformycin A, while relatively stable in crystalline form, decomposes in solution by an intramolecular SN_2 reaction to the two anhydroformycins prepared by J. Zemlicka[21]. It exhibits a strong blue fluorescence in UV light, UV absorption at 305 nm and downfield shifting in the NMR spectrum of many of the standard formycin A and B peaks, including C_5-H to 8.64 ppm, iPrH to 1.25 and 1.45 ppm, and the range of the ribose peaks from 3.3-5.44 to 4.4-5.67, so that its NMR spectrum conforms to that given by Zemlicka[21] for the N_4-C_5' intramolecular anhydride of formycin A. Formation of anhydroformycins appeared to be favored by higher temperature, as a 35% yield could be recovered if the reaction was was run at room temperature as opposed to 12% at 0°C. A second fluorescent spot could be demonstrated by TLC, presumably the N_2-C_5 anhydroformycin synthesized by Zemlicka[21]. The deblocked iodoformycin A is stable. The following procedure was used to prepare enough iodoformycin A for characterization and biological testing after deblocking.

A suspension of 1.9 g (4.2 mmol) of methyltriphenoxy phosphonium iodide in 30 ml of dry THF was added dropwise to a stirred suspension of compound 2 (450 mg, 1.5 mmol) in 20 ml of dry THF at 0° under nitrogen. After stirring 1 1/2 hours an additional 0.4 g of methyltriphenoxyphosphonium iodide was added. The mixture was then stirred for 3 hours, while it was allowed to warm to room temperature, and filtered from insoluble anhydroformycins (12%). The filtrate was evaporated to dryness under vacuum and the residue taken up in methylene chloride (125 ml), washed with dilute aqueous sodium bisulfite and water, dried over $MgSO_4$ and concentrated to a colorless

oil. Purification by flash chromatography (CH_2Cl_2-EtOH, 20:3) yielded two similar materials. The more polar (R_f 0.47), crystallized slowly from a minimal quantity of EtOH at -8°C, to give an analytically pure sample of **3** (50 mg, 8.2%), m.p. 149°C (dec); R_f(A), 0.42; UV(EtOH): λ_{max} 295 nm (10,700); IR (KBr): 1620 cm^{-1} (NH_2), 1170 cm^{-1} (C-O-C); NMR(DMSO-d_6): δ 1.33 and 1.52 (2s, 6H, CH_3 of ketal), 3.3 (m, H_2O and C_5'-H), 4.19 (m, 1H, C_4'-H), 4.86 (m, 1H, C_2'-H or C_3'-H), 5.27 (m, 1H, C_1'-H), 5.45 (m, 1H, C_3'-H or C_2'-H), 7.33 (br s, 2H, NH_2), 8.24 (s, 1H, C_5-H).
Anal. Calc'd for $C_{13}H_{16}N_5O_3I$: C, 37.43; H, 3.87; N, 16.79, I, 30.42. Found: C, 37.28; H, 3.86; N, 16.63; I, 30.22.

The slower moving component (R_f 0.33), isolated in 40% yield from the flash chromatogram, appeared to be a 5'-methylphosphonate of formycin A (**11**). On the basis of its hydrolysis product described below, its ^1H NMR shifts were assigned as follows: NMR(DMSO-d_6): δ 1.37, 1.51, 1.57 and 1.80 (4s, 9H, CH_3 of ketal and CH_3-P), 4.34 (m, 4.34 (m, 3H, C_2'-H, C_3'-H, C_4'-H), 5.02 (br s, 1H, C_1-H), 5.43 (m, 2H, C_5'-H), 6.84-7.28 (m, 2H, NH_2), superimposed on 7.24 (s, 5H, Ar-H), 8.29 (s, 1H, C_5-H).

After deblocking in 90% formic acid the product was identified as the 5'-methylphenoxyphosphonate of formycin A (**12**). UV(EtOH): λ_{max} 296 nm (9600); (pH 1): λ_{max} 302 nm (8200); IR(KBr): 1650 cm^{-1} (NHCO), 1210 and 700 cm^{-1} (P-O-C^{25}); NMR(DMSO-d_6): δ 1.48, 1.77 (2s, 3H, CH_3-P), 3.63-4.48 (m, 4H, ribose H), 4.47-5.75 (m, 2H, ribose H), 7.22 (s, 7H, Ar-H and NH_2), 8.45, (s, 1H, C_5-H).
Anal. Calc'd for $C_{17}H_{20}N_5O_6P$: C, 48.46; H, 4.78; N, 16.62; O, 22.78; P, 7.35. Found: C, 48.75; H, 4.97; N, 16.39; O, 22.61; P, 7.62.

7-Amino-3-(5'-iodo-5'-deoxy-β-D-ribofuranosyl)-pyrazolo-(3,4-d)-pyrimidine (**4**).

Compound **3**, 65 mg (0.16 mmol), was deblocked in 0.5 ml of 90% formic acid at 0°C. TLC indicated that the reaction had proceeded to completion after 36 hours. The product was purified on preparative TLC plates using CH_2Cl_2-MeOH, 10:1 + 0.5% HCOOH as solvent. Analytically pure **4** (38 mg, 65%) was obtained as the formate salt from EtOH. R_f(B) 0.34; UV(EtOH): λ_{max} 295 nm (10,200); IR(KBr): 1620 cm^{-1} (NH_2), no (C-O-C) at 1190 cm^{-1}; NMR(DMSO-d_6): δ 2.84-3.65 (m, 2H, C_5'-H on OH-H_2O peak), 3.91 (m, 1H, C_4'-H), 4.18 (m, 1H, C_3'-H), 4.71 (m, 1H, C_2'-

H), 5.00 (m, 1H, $C_{1'}$-H), 5.18 (br s, 1H, $C_{5'}$-OH), 7.36 (br s, 2H, NH_2), 8.18 (s, 1H, C_5-H), 8.20 (s, 1H, HCOO-).
Anal. Calc'd for $C_{10}H_{12}N_5O_3I \cdot 1.5\ HCOOH \cdot 0.25\ H_2O$: C, 30.65; H, 3.47; N, 15.54; I, 28.16. Found: C, 30.67; H, 3.85; N, 15.55; I, 28.42.

3-(2',3'-Di-O-isopropylidine-β-D-ribofuranosyl)-pyrazolo-(3,4-d)-pyrimidone (6).

Formycin B (0.8 g, 3 mmol) was converted to its isopropylidine derivative in acetone solution in the presence of p-toluenesulfonic acid (0.64 g, 3.7 mmol) and triethylorthoformate (2.1 ml, 14 mmol). Recrystallization from water yielded two crops of analytically pure, white hydrophobic leaflets (0.62 g, 68%). M.p. 264.5-266°C; R_f(A) 0.44; UV(EtOH): λ_{max} 273 nm (8000); IR(KBr): 1690 cm^{-1} ($NHCO^{25}$), 1190 cm^{-1} (C-O-C); NMR(DMSO-d_6): δ 1.32, 1.56 (2s, 6H, CH_3 of ketal), 3.50 (s, 2H, $C_{5'}$-H), 4.04 (m, 2H, $C_{3'}$-H, $C_{4'}$-H), 4.69-4.89 (m, 1H, $C_{2'}$-H), 5.10 (m, $C_{1'}$-H or N_1-H), 7.89 (s, 1H, C_5-H).
Anal. Calc'd for $C_{13}H_{16}N_4O_5$: C, 50.65; H, 5.23; N, 18.17. Found: C, 50.80; H, 5.47; N, 17.92.

3-(5'-Iodo-5'-deoxy-2',3'-di-O-isopropylidine-β-D-ribofuranosyl)-pyrazolo-(3,4-d)-7-pyrimidone (7).

Compound 6 (300 mg) was iodinated with 2 equivalents (650 mg, 1.5 mmol) of methyltriphenoxyphosphonium iodide in dry THF at -20°C, and the suspension allowed to warm to room temperature over 3 hours with stirring. The solvent was evaporated and the residue taken up in methylene chloride (200 ml), washed briefly with minimal amounts of aqueous $Na_2S_2O_3$ and water, evaporated, and the residue washed with hexane and dried. Solid 7 fractions were purified by flash chromatography (CH_2Cl_2-EtOH, 10:1) to yield 270 mg (67%). An analytical sample recrystallized from acetone melted at 152°C (dec), R_f(A) 0.63; UV(MeOH): λ_{max} 270 nm (7200); NMR(DMSO-d_6): δ 1.27, 1.37 and 1.49 (3s, 6H, CH_3 of ketal), 4.14 (m, 1H, $C_{4'}$-H), 4.82 (s, 4H, $C_{5'}$-H, $C_{3'}$-H, $C_{2'}$-H), 5.24 (s, 1H, $C_{1'}$-H), 7.81 (s, 1H, C_5-H).
Anal. Calc'd for $C_{13}H_{15}N_4O_4I$: C, 37.34; H, 3.62; N, 13.40. Found: C, 37.34; H, 3.81; N, 13.17.

3-(5'-Iodo-5'-deoxy-β-D-ribofuranosyl)-pyrazolo-(3,4-d)-7-pyrimidone (8).

To 100 mg of compound 7 (0.,24 mmol) there was added 0.5 ml of 90% formic acid, and the mixture stored overnight at 8°C. Co-

evaporation with MeOH (3x) to remove excess formic acid, neutralization to pH 8 with methanolic NH_3, removal of solvent and recrystallization from EtOH yielded 55 mg (61%) of white crystalline **8**, suitable for analysis. M.p. 184°C (dec), R_f(B) 0.63; UV(EtOH): λ_{max} 271 nm (8200); NMR(DMSO-d_6): δ 3.03-3.70 (m, OH-H_2O and $C_{5'}$-H overlap), 3.89 (m, 1H, $C_{4'}$-H), 4.09 (m, 1H, $C_{3'}$-H), 4.60 (tr, 1H, $C_{2'}$-H), 4.98 (d, 1H, $C_{1'}$-H), 5.29 (br s, 2H, $C_{5'}$-OH), 7.90 (s, 1H , C_5-H). Anal. Calc'd for $C_{10}H_{11}N_4O_4I$: C, 31.76; H, 2.93; N, 14.82; I, 33.56. Found: C, 31.66; H, 3.27; N, 14.82; I, 33.39.

7-Amino-3-(5'-chloro-5'-deoxy-β-D-ribofuranosyl)-pyrazolo-(3,4-d)-pyrimidine (9).

Formycin A **1** (1.0 g, 3.76 mmol) was chlorinated by the procedure of Kikugawa and Ichino[26]. Compound **1** was added to a stirred mixture of HMPT (8 ml) and thionyl chloride (15 ml) under nitrogen at room temperature for 24 hours. The product was purified on a Dowex 50 (H+) column and eluted with 4N NH_4OH to yield 850 mg (81%) of white crystalline product. A sample for analysis was recrystallized from water. R_f(B) 0.30; UV(H_2O): λ_{max} 295 nm (10,400); NMR(DMSO-d_6): δ3.52(br s, 2H, $C_{2'}$-OH, $C_{3'}$-OH, D_2O labile), 3.91-4.31 (m, 2H, $C_{5'}$-H), 4.06-4.25 (m, 1H, $C_{4'}$-H), 4.27-4.47 (m, 1H, $C_{3'}$-H), 4.71-4.93 (m, 1H, $C_{2'}$-H), 5.24 (m, 1H, $C_{1'}$-H) superimposed on 5.30 (br s, 1H, N_1-H, D_2O labile), 7.58 (br s, 2H, NH_2, D_2O labile), 8.40 (s, 1H, C_5-H). Anal. Calc'd for $C_{10}H_{11}N_4O_4Cl \cdot 1.25\ H_2O$: C, 38.97; H, 4.74; N, 22.72; Cl, 11.50. Found: C, 38.99; H, 4.61; N, 23.04; Cl, 11.76

3-(5'-Chloro-5'-deoxy-β-D-ribofuranosyl)-pyrazolo-(3,4-d)-7-pyrimidone (10).

Using a modified procedure of Umezawa et al[27,28], chloroformycin A **9** (500 mg, 1.6 mmol) was deaminated to chloroformycin B by stirring at room temperature overnight with a solution of 1.5 g of sodium nitrite in a mixture of 10 ml of glacial acetic acid and 20 ml of water. After 21 hours the solution was cooled to 0°, neutralized to pH 8 with concentrated NH_4OH, and evaporated. The yellow solid product was isolated, sublimed in vacuo to separate it from salts, evaporated from ethanol solution onto silica gel beads and loaded into a pre-packed column to be developed with CH_2Cl_2-MeOH, 60 : 13. An analytical sample recrystallized from water melted at 191°C dec. R_f(B) 0.45. UV(EtOH): λ_{max} 272 nm (9300); NMR(DMSO-d_6): δ 3.05-3.90 (br s, OH-H_2O), 3.67-3.88 (ddd, 2H, $C_{5'}$-H; $J_{4',5'a}$ = 4.5 Hz, $J_{4',5'b}$ =

6.8 Hz, J_{gem} = 11.4 Hz), 3.94-4.04 (td, 1H, $C_{4'}$-H; $J_{3',4'}$ = 4.8 Hz, $J_{4',5'}$ = 6.7 Hz), 4.16 (t or dd, 1H, $C_{3'}$-H; $J_{2',3'}$ = 5.2 Hz, $J_{3',4'}$ = 5.2 Hz), 4.55 (t, 1H, $C_{2'}$-H; $J_{1',2'}$ = 5.4 Hz, $J_{2',3'}$ = 5.3 Hz), 4.99 (d, 1H, $C_{1'}$-H; $J_{1',2'}$ = 5.5 Hz), 7.89 (s, 1H, C_5-H).
Anal. Calc'd for $C_{10}H_{11}N_4O_4Cl \cdot 1/8\ H_2O$: C, 41.57; H, 3.92; N, 19.39; Cl, 12.27. Found: C, 41.82; H, 4.03; N, 19.59; Cl, 11.87.

7-Amino-3-(5'-bromo-5'-deoxy-β-D-ribofuranosyl)-pyrazolo-(3,4-d)-pyrimidine (13).

This compound was prepared by the method of Kikugawa and Ichino[26], using thionyl bromide in HMPT solution. Yields of analytically pure material eluted from a preparative TLC plate (silica gel GF, Analtech) were of the order of 20%. UV(EtOH)): λ_{max} 294 (10,800); NMR(DMSO-d_6): δ 3.17-3.50 (br s, OH-H_2O), 3.55-3.85 (2m, 2H, $C_{5'}$-H), 3.99-4.10 (m, 1H, $C_{4'}$-H), 4.22 (m, 1H, $C_{3'}$-H), 4.67 (t, 1H, $C_{2'}$-H), 5.22 (d, 1H, $C_{1'}$-H), 5.25 (br.s, 1H, N_1-H), 7.34 (br.s, 1-2H, NH_2), 8.28 (s, 1H, C_5-H).
Anal. Calc'd for $C_{10}H_{12}N_5O_3Br \cdot 5/8\ H_2O$: C, 35.19; H,3.91; N, 20.52; Br, 23.41. Found: C, 35.59; H, 3.90; N, 20.47; Br, 23.01.

3-(5'-Bromo-5'-deoxy-β-D-ribofuranosyl)-pyrazolo-(3,4-d)-7-pyrimidone (14).

Compound **14** was prepared from Formycin B (**2**) by the method of Kashem et al[22]. Formycin B (0.8 g) in 60 ml of dry pyridine was cooled to 0° and treated with 1.72 g of triphenylphosphine and 1.08 g of carbon tetrabromide in three portions and stirred for 15 minutes at 70-80° C . Methanol (10 ml) was added to decompose the reagent and solvents removed at 40° under vacuum. The residue was chromatographed on a preparative silica gel plate and developed twice with CH_2Cl_2-EtOH (20:3). 200 mg of analytically pure material was eluted from the silica and recovered as a fluffy white powder that decomposed above 200° without melting. (R_f was about 0.15). UV(EtOH): λ_{max} 272 (6000); NMR(DMSO-d_6): δ 3.54 (br.s, >4H, 2'-OH, 3'-OH, H_2O and $C_{5'}$-H overlap), 4.00-4.08 (m, 1H, $C_{4'}$-H), 4.16 (m, 1H, $C_{3'}$-H), 4.60 (m, 1H, $C_{2'}$-H), 5.00 (m, 1H, $C_{1'}$-H), 7.93 (s, 1H, C_5-H), 12.83 (br.s, 1H, Ar-OH).
Anal. Calc'd for $C_{10}H_{11}N_4O_4Br \cdot 1/4\ H_2O$: C, 36.26; H, 3.35; N, 16.92; Br, 24.13. Found: C, 36.05; H, 3.59; N, 16.32; Br, 24.45.

Alternatively, compound **14** could be made by deaminating **13** by a modified procedure of Umezawa[27,28] in the same manner as compound **9**, using sodium nitrite and glacial acetic acid with a little water. Yields were similar.

Biological Methods

MTAPase activity was determined by the spectrophotometric assay described in ref. 10. Growth inhibition studies on the L5178Y and L1210 murine leukemia cells were carried out as previously described[29].

RESULTS

Biological Studies

The 5'-halogenated derivatives of formycin A, 5'-chloro-5'-deoxyformycin (5'-ClF), 5'-bromo-5'-deoxyformycin (5'-BrF). and 5'-iodo-5'-deoxyformycin (5'-IF), each inhibit MTAPase, partially-purified[10] from murine Sarcoma 180 cells, with K_i values in the 10^{-7} M range (Table 1).

Double-reciprocal plots indicate that these compounds act as competitive inhibitors of MTAPase with respect to MTA (see Figure 1 for the data with 5'-ClF).

It should be noted that the 5'-halogenated formycins are some 2 orders of magnitude more potent than 5'-deoxy-5'-methylthiotubercidin

Table 1. K_i values of 5'-halogenated formycins with MTAPase from Sarcoma 180 cells

5'-Halogenated Formycin A	K_i (10^{-6} M)
5'-Chloro-5'-deoxyformycin A	0.50
5'-Bromo-5'-deoxyformycin A	0.21
5'-Iodo-5'-deoxyformycin A	0.17

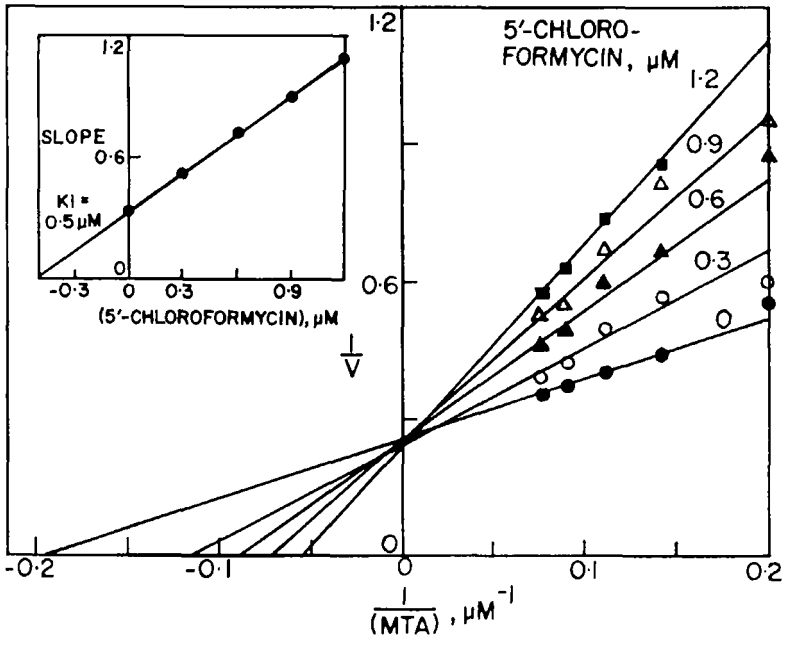


Figure 1

(5'-deoxy-5'-methylthio-7-deazaadenosine), the only other inhibitor of mammalian MTAPase reported to date[30,31]. Also of significance is the fact that the K_i values for the 5'-halogenated formycins are approximately 8 to 24 fold lower than the K_m value of the natural substrate, MTA, for the enzyme (4 M)[10].

To demonstrate that the 5'-halogenated formycins can act as inhibitors of MTAPase in intact cells, it was examined whether they could block the growth inhibitory effects of MTA analogs which require MTAPase for activation. Examples of such compounds are the 5'-halogenated adenosine derivatives 5'-bromo-5'-deoxyadenosine and 5'-iodo-5'-deoxyadenosine, which act as substrates of MTAPase from both human[29] and murine[32] sources and have growth-inhibitory activity. Because these compounds have at least 20 fold more growth inhibitory activity against MTAPase-containing lines than those cell lines that lack MTAPase, it is probable that one of the products of the reaction of these 5'-halogenated adenosines with MTAPase is primarily responsible for causing the growth inhibition[29]. Since one product of

Table 2. Ability of 5'-halogenated formycins to protect L5178Y cells from the growth inhibitory actions of 5'-iodoadenosine.

MTAPase Inhibitor (10^{-4} M)	Percent Inhibition of Cell Growth: 5'-Iodoadenosine 1.0×10^{-5} M	5'-Iodoadenosine 3.0×10^{-6} M
None	100	85
5'-Chloroformycin A	71	23
5'-Bromoformycin A	38	17
5'-Iodoformycin A	10	5

the reaction, adenine, is innocuous to cells, the other product, namely the 5-halogenated ribose-1-phosphate, is implicated as the active cytotoxic metabolite[29]. This idea is further supported by the finding that 5'-halogenated inosines, which are substrates of PNP yielding hypoxanthine and a 5-halogenated ribose-1-phosphate[20], are growth inhibitory to cells that contain high levels of PNP activity[29]. Thus 5-halogenated ribose-1-phosphates, *e.g.* 5-iodoribose-1-phosphate, appear to be the active metabolite of both the 5'-halogenated adenosines and inosines[29,33].

On this basis it would be expected that inhibition of MTAPase could limit the formation of the 5-halogenated ribose-1-phosphate metabolite from the 5-halogenated adenosines, and thereby protect cells from the growth inhibitory effects of these compounds (Table 2). Similarly, a blockade of PNP should protect cells from the toxic effects of 5'-halogenated inosines as shown in Table 3.

5'-ClF, 5'-BrF, and 5'-IF at 10^{-4} M protect murine L5178Y leukemia cells, which contain MTAPase (1.1 nmol units/mg prot) from the growth-inhibitory actions of 5'-iodo-5'-deoxyadenosine. 5'-Chloro-5'-deoxyformycin B and 5'-iodo-5'-deoxyformycin B (Compounds **10** and **8**, respectively), which have been shown to be competitive inhibitors of PNP with K_i values in the 10^{-5} M range[20], protect murine L1210 leukemia cells, which have relatively high PNP activity (169

Table 3. Ability of 5'-halogenated formycin B derivatives to protect L1210 murine leukemia cells from the growth inhibitory effects of 5'-iodoinosine.

PNP Inhibitor (3×10^{-5} M)	Percent Inhibition of Cell Growth: 5'-Iodoinosine 1.0×10^{-5} M	5'-Iodoinosine 3.0×10^{-6} M
None	65	31
Formycin B	65	29
5'-Chloroformycin B	56	14
5'-Iodoformycin B	26	0

nmol units/mg prot), against the growth-inhibitory actions of 5'-iodo-5'-deoxyinosine (Table 3).

These data suggest that 1) the 5'-halogenated formycin A derivatives can inhibit MTAPase in intact cells, and 2) support the hypothesis that 5-halogenated ribose-1-phosphates are the active metabolites of both the 5'-halogenated adenosines and inosines.

Acknowledgements: This work has been supported by USPHS grants CA 13943, CA 20892, CA 31650, CA 34228, and American Cancer Society Grant CH-266. We are grateful to Drs. Robert E. Parks, Jr. and Paul Calabresi for their encouragement during the course of this investigation. We also thank Dr. James van Epp for assistance with ^{1}H NMR spectra.

REFERENCES

1. A.E.Pegg and H.G.Williams-Ashman, J.Biol.Chem. 244, 682 (1969).

2. A.E.Pegg and H.G.Williams-Ashman, Arch. Biochem. Biophys. 137, 156 (1970).

3. A.E.Pegg and H.G.Williams-Ashman, Biochem. J. 115, 241 (1969).

4. P.S.Backlund,Jr. and R.A.Smith, J.Biol.Chem. 256, 1533 (1981).

5. P.S.Backlund,Jr., C.P.Chang, and R.A.Smith, J. Biol. Chem. 257, 4196 (1982).

6. P.S.Backlund,Jr. and R.A.Smith, Biochem. Biophys. Res. Commun. 108, 687 (1982).

7. P.C. Trackman and R.H. Abeles, J. Biol. Chem. 258, 6717 (1983).

8. D.A.Carson, E.H.Willis, and N.Kamatani, Biochem. Biophys. Res. Commun.112, 391 (1983).

9. T.M.Savarese, L.Y.Ghoda, D.L.Dexter, and R.E.Parks,Jr., Cancer Res. 43, 4699 (1983).

10. T.M.Savarese, G.W.Crabtree, and R.E.Parks,Jr., Biochem. Pharm. 30, 189 (1981).

11. N. Kamatani and D.A. Carson, Biochim. Biophys. Acta 675, 344 (1981).

12. H.G.Williams-Ashman, J.Seidenfeld, and P.Galletti, Biochem. Pharm. 31, 272 (1982).

13. G.Wolberg, T.P.Zimmerman, C.J.Schmitges, G.S.Duncan, and R.D.Deeprose, Biochem. Pharm. 31, 2203 (1982).

14. M.K.Riscoe, P.A.Tower and A.J.Ferro, Biochem. Pharm. 33, 3639 (1984).

15. R.L.Pajula, A.Raina, and T.Eloranta, Eur. J. Biochem. 101, 619 (1979).

16. H.Hibasami, R.T.Borchardt, S.Y.Chen, J.K.Coward, and A.E.Pegg, Biochem. J. 187, 419 (1980).

17. A.J. Ferro, A.A.Vandenbark, and M.R. MacDonald, Biochem. Biophys. Res. Commun. 100, 523 (1981).

18. I.H.Fox, T.D.Palella, D.Thompson, and C.Herring, Arch. Biochem. Biophys. 215, 302 (1982).

19. M.R.Sheen, B.K.Kim, and R.E.Parks,Jr., Molec. Pharmac. 4, 293 (1968).

20. J.D.Stoeckler, C.Cambor, V.Kuhns, S.H.Chu, and R.E.Parks,Jr., Biochem. Pharm.31, 1723 (1982).

21. J. Zemlicka, J.Am.Chem.Soc., 97, 5896 (1975).

22. A.Kashem, M.Anisuzzaman and R.L.Whistler, Carbohydrate Res., 61, 511, (1978).

23. J.Tomasz, in Nucleic Acid Chemistry; Part II; (L.B.Townsend and R.S.Tipson, eds.) John Wiley & Sons, New York, 1978, p.765.

24. J.P.H.Verheyden and J.G.Moffatt, J.Org.Chem. 15, 2868 (1978).

25. D.H.Williams and I.Fleming, Spectroscopic methods in Organic Chemistry, 2nd Ed., McGraw-Hill Book Co. (UK) Ltd., New York, N.Y. 1973, pp. 42-65.

26. K.Kikugawa and M.Ichino, Tetrahedron Lett. 2, 87 (1971).

27. H.Umezawa, T.Sawa, Y.Fukagama, G.Koyama, M.Murase, H.Hamada and T.Takeuchi, J.Antibiotics(Tokyo), 18A, 178 (1965).

28. T.Takeuchi, J.Iwanaga, T.Aoyagi and M.Umezawa, J. Antibiotics, (Tokyo), 19A, 286 (1966).

29. T.M.Savarese, S.H.Chu, M.Y.Chu, and R.E.Parks,Jr., Biochem. Pharm. 34, 361 (1985).

30. J.K.Coward, N.C. Motola, and J.D. Moyer, J.Med. Chem. 20, 500 (1977).

31. A.J. Ferro, M.K. Riscoe, and M.W. White, In: Biochemistry of S-Adenosylmethionine and Related Compounds (E. Usdin, R.T. Borchardt and C.R. Creveling, eds.) MacMillan, London, p. 693 (1982).

32. R.E.Parks,Jr., T.M.Savarese, and S.H.Chu, in: New Approaches to the Design of Antineoplastic Agents (T.I.Kalman and T.J.Bardos, eds.) Elsevier North Holland, N.Y. p. 141 (1982).

33. H.S.Choi, J.D. Stoeckler, and R.E. Parks, Jr., J. Biol. Chem. (in press).

Received October 14, 1985.