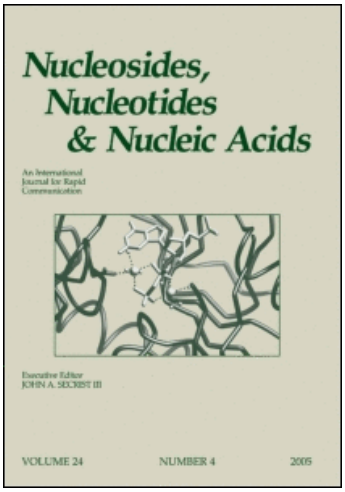

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Synthesis and Biological Activity of C-Acyclic Nucleosides of Imidazo [1,5-a]-1,3,5-Triazines

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SYNTHESIS AND BIOLOGICAL ACTIVITY OF C-ACYCLIC NUCLEOSIDES OF IMIDAZO [1,5-a]-1,3,5-TRIAZINES

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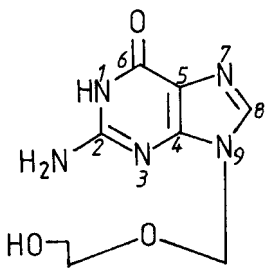
Summary

C-acyclic nucleoside analogues of inosine and guanosine 8-[(RS)-2,3-dihydroxypropyl]imidazo[1,5-a]-1,3,5-triazin-4(3H)-ones 6a, c, d were synthesized. The route involved the cyclization-rearrangement of 5-acylamino-5-allyl-6-amino-4,5-dihydropyrimidin-4-ones 4a-c to 8-allylimidazo[1,5-a]-1,3,5-triazin-4(3H)ones 5a-c. 5a was transformed selectively into 5d by reductive desulfurization with highly deactivated Raney nickel. The poorly soluble compounds 5b and 5c were converted to N-2-acetylated 5f and 5g. Osmium tetroxide hydroxylation of 5d, f, g gave 6a, c, d. None of the newly synthesized C-acyclic nucleoside derivatives showed an appreciable antiviral or antitumor cell activity.

The promising antiviral activity of several nucleoside analogues in which the ribose moiety has been replaced by an acyclic moiety (1,2,3)[1-3] has given a strong incentive to the search of new congeners.[4-8] Not very many C-nucleosides of this type have been reported so far.[4,8,9]



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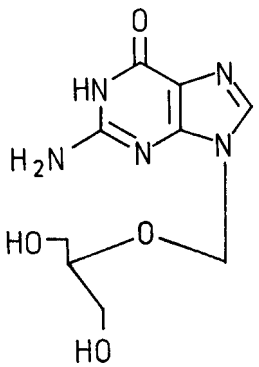

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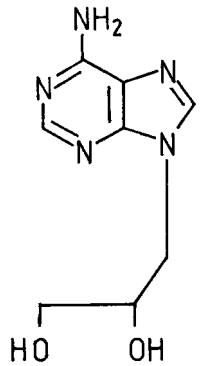
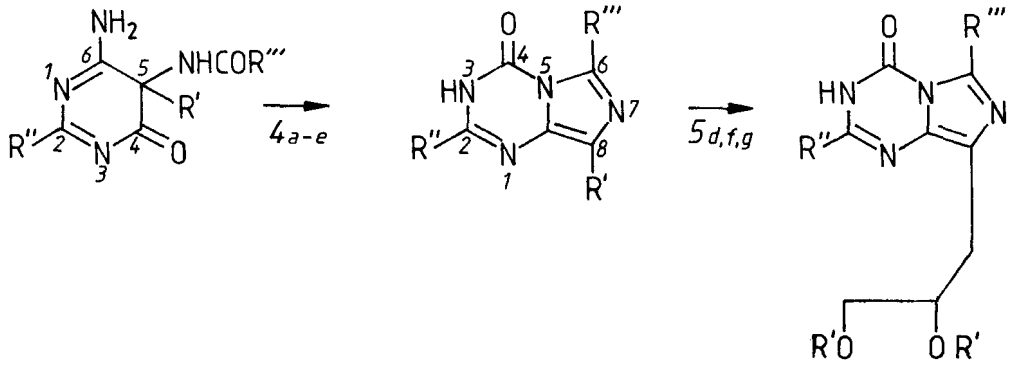


3

We have undertaken the synthesis of C-acyclic nucleosides containing an isomeric to purine imidazo[1,5-a]-1,3,5-triazine ring system. In this paper we describe the synthesis and biological activity of the 8-[(RS)-2,3-dihydroxypropyl]derivatives analogous to inosine and guanosine.

Chemistry[10]

The synthetic route we used for the preparation of the series of 8-[(RS)-2,3-dihydroxypropyl]imidazo[1,5-a]-1,3,5-triazin-4(3H)-ones and several related compounds is outlined in Scheme I.



4

	R'	R''	R'''
a	$CH_2CH=CH_2$	SH	CH_3
b	$CH_2CH=CH_2$	NH_2	H
c	$CH_2CH=CH_2$	NH_2	CH_3
d	$CH(CH_3)_2$	SH	CH_3
e	$CH_2C_6H_5$	SH	CH_3

5

	R'	R''	R'''
a	$CH_2CH=CH_2$	SH	CH_3
b	$CH_2CH=CH_2$	NH_2	H
c	$CH_2CH=CH_2$	NH_2	CH_3
d	$CH_2CH=CH_2$	H	CH_3
e	$CH_3CH_2CH_2$	H	CH_3
f	$CH_2CH=CH_2$	$NHCOCH_3$	H
g	$CH_2CH=CH_2$	$NHCOCH_3$	CH_3
h	$CH(CH_3)_2$	SH	CH_3
i	$CH_2C_6H_5$	SH	CH_3

6

	R'	R''	R'''
a	H	H	CH_3
b	H	NH_2	CH_3
c	H	$NHCOCH_3$	H
d	H	$NHCOCH_3$	CH_3
e	$COCH_3$	$NHCOCH_3$	H
f	$COCH_3$	$NHCOCH_3$	CH_3

+ The listing of R'' conveniently indicate substitution but do not necessarily indicate the favored tautomeric form. Some details of the latter will be published elsewhere.

SCHEME I

The approach was based upon the cyclization - rearrangement reaction of 5-R', 5-acylamino-6-amino-4,5-dihydropyrimidin-4-ones 4, leading to imidazo[1,5-a]-1,3,5-triazin-4(3H)-ones in which substituent R' is located at C-8, position analogous to N-9 of purines.[11] An introduction of 2,3-dihydroxypropyl moiety was effected by using an allyl grouping as R' substituent and subsequent hydroxylation of the double bond.

Treatment of 5-acetylamino-5-allyl-6-amino-4,5-dihydro-2(3H)-thioxopyrimidin-4-one (4a) with chlorotrimethylsilane and hexamethyldisilazane in pyridine under reflux furnished a thioxanthine analogue, 8-allyl-6-methyl-2(1H)-thioxoimidazo[1,5-a]-1,3,5-triazin-4(3H)-one 5a in 84 % yield. 8-Allyl substituted imidazo[1,5-a]-1,3,5-triazin-4-one analogues of guanines (5b and 5c) have already been reported by others.[12] As for 5b and 5c examination of the ^{1}H NMR spectrum of 5a revealed a crucial signal for the methylene protons of the allyl group centered at δ 3.20, indicative of C-substitution. Transformation of 5a to the hypoxanthine analogue 5d was initially performed by deactivated Raney nickel desulfurization in aqueous ammonia.[11] Under these conditions, however, the allylic side chain underwent simultaneous saturation to give a by-product, 5e, in over 20 % yield. The presence of 5e in the 5d preparation could be detected by ^{1}H NMR and TLC, but it was rather difficult to remove 5e from the mixture. In efforts to improve the yield of the desired 8-allyl compound 5d we found that formation of the by-product could be completely suppressed by performing desulfurization in boiling ethanol, in the presence of Raney nickel deactivated by prolonged heating in boiling water. Under these conditions 5d was obtained in 59 % yield.

In applying the hydroxylation procedure for the formation of the C-acyclonucleoside analogues, one had to take into account the relatively low chemical stability of the imidazo[1,5-a]-1,3,5-triazinones. Generally, osmium tetroxide gave the best results. It turned out, however, that individual derivatives differed distinctly from each other with respect to stability toward the reagents used to cleave the osmate esters. Three different reaction conditions were evaluated to determine the most advantageous ones. These were : A) osmium tetroxide, equivalent amount as 0.5 % solution in t-butanol, catalytic amount of pyridine, solvent t-butanol, room temperature, cleavage of the osmate ester with 40 % sodium hydrogen sulfite;[13] B) conditions as in A, cleavage with SH^- by 10 % aqueous thioacetamide;[14] C) osmium tetroxide, catalytic amount as 0.5 % solution in

t-butanol, sodium chlorate, 4 eq., solvent methanol-water (1:1), room temperature, diol liberated by sodium chlorate during the reaction.[15]

In the case of the hypoxanthine analogue 5d formation of the crystalline diol 6a could have been accomplished by any of the approaches (A, 85 %; B, 44 %; C, 80 %). Method A was superior in terms of yield and easiness. However, this procedure was not applicable to the hydroxylation of guanine analogues 5b and 5c, because upon treatment with sodium hydrogen sulfite they underwent degradation to UV-transparent products. Cleavage of osmate ester with hydrogen sulfide, according to procedure B, could be achieved, but low solubility of both the substrate 5c and the product 6b made impossible the isolation of the latter at a preparative scale. To improve solubility, 5b and 5c were first transformed into N-2-acetyl derivatives 5f and 5g and then subjected to hydroxylation under the conditions of method B. The diols 6c and 6d that were formed this way could be isolated in a pure form only by conversion into respective crystalline triacetyl derivatives 6e and 6f and subsequent O-deacetylation with potassium carbonate in anhydrous methanol. The overall yield was good (63 %) for 6-unsubstituted 6c but low (26 %) for 6-methyl congener 6d. The application of method C allowed to improve the yield of 6d up to 63 % under a much simpler work-up procedure. Unfortunately, the above method could not be used for the preparation of 6c. Both 5f and 6c were found to decompose in the presence of sodium chlorate.

The cyclization-rearrangement reaction allowed to introduce into position C-8 of imidazo[1,5-a]-1,3,5-triazin-4-one system more bulky substituents than allyl. 8-Isopropyl-6-methyl-2(1H)thioxoimidazo[1,5-a]-1,3,5-triazin-4(3H)-one 5h and the corresponding 8-benzyl derivative 5i were formed readily in high yields.

Biological activity. Compounds 5a,d,f,g,h,i and 6a,d,c were evaluated for antiviral activity in a variety of virus assay systems (Table 1), but, under conditions where acyclovir (1) proved specifically active against herpes simplex virus (types 1 and 2) and (S)-DHPA (3) and ribavirin exhibited the expected[3,16] broad-spectrum antiviral activity, none of the test compounds effected an appreciable inhibition of virus-induced cytopathogenicity.

Similarly, no marked antitumor cell activity was observed with any of the test compounds when evaluated for their inhibitory effects on the growth of four well-established tumor cell lines (Table 2). Of all com-

Table 1. Antiviral Activity of Nucleoside Analogues

Compound	Minimum inhibitory concentration[a] (μg/mL)							
	Primary rabbit kidney cells			Vero cells			HeLa cells	WI-38 cells
	Herpes simplex virus type 1 (KOS) & type 2 (G)	Vaccinia virus	Vesicular stomatitis virus	Para-influenza virus type 3	Reo virus type 1	Sindbis & Semliki Forest virus	Coxsackie B4 virus & polio virus type 1	Rhinovirus type 1A & type 9
5a	>40 (40)[b]	>40	>40	>200 (200)	>200	>200	>200 (200)	>100 (100)
5d	>100 (100)	>100	>100	>200 (200)	>200	>200	>400 (>400)	>200 (200)
5f	>200 (200)	>200	>200	>200 (200)	>200	>200	>400 (>400)	≥200 (400)
5g	>200 (200)	>200	>200	>200 (200)	>200	>200	>400 (>400)	>200 (400)
5h	>200 (200)	>200	>200	>200 (200)	>200	>200	300 (>400)	>200 (200)
5i	150 (200)	150	150	>100 (100)	>100	>100	200 (200)	>100 (100)
6a	>400 (>400)	>400	>400	>400 (>400)	>400	>400	>400 (>400)	≥300 (>400)
6d	>200 (200)	>200	>200	>200 (200)	>200	>200	>200 (200)	>400 (>400)
6c	>400 (400)	>400	>400	>200 (200)	>200	>200	>400 (>400)	>400 (>400)
1 (Acyclovir)	0.07 (>400)	150	>400	>100 (100)	>100	>100	>400 (>400)	>400 (>400)
3 [(S)-DHPA)]	>400 (>400)	70	20	20 (>400)	20	>400	>400 (>400)	>400 (>400)
Ribavirin	>400 (>400)	20	150	40 (>400)	70	30	20 (>400)	100 (>400)

[a] Required to reduce virus-induced cytopathogenicity by 50 %.
[b] In parentheses are the minimum cytotoxic concentrations (μg/mL), causing a microscopically detectable alteration of normal cell morphology. WI-38 cells correspond to human diploid fibroblasts.

Table 2. Antitumor Cell Activity of Nucleoside Analogues

Compound	50 % inhibitory concentration[a] (μg/mL)			
	Murine leukemia L1210 cells	Murine mammary carcinoma FM3A cells	Human B-lymphoblast Raji cells	Human T-lymphoblast Molt/4F cells
5a	339 ± 20	327 ± 30	166 ± 18	305 ± 34
5d	308 ± 68	376 ± 84	305 ± 10	333 ± 63
5f	> 1000	> 1000	676 ± 200	> 1000
5g	325 ± 16	> 1000	436 ± 95	424 ± 202
5h	318 ± 15	242 ± 40	163 ± 66	258 ± 63
5i	231 ± 67	174 ± 85	80 ± 29	192 ± 62
6a	> 1000	> 1000	> 1000	> 1000
6d	> 1000	> 1000	> 1000	> 1000
6c	299 ± 15	359 ± 38	321 ± 33	249 ± 61
1 (Acyclovir)	40 ± 6	71 ± 44	798 ± 210	> 1000
3 [(S)-DHPA)]	198 ± 89	45 ± 8	268 ± 13	302 ± 93
Ribavirin	5 ± 0.5	2.4 ± 0.1	55 ± 18	32 ± 16

[a]Required to reduce tumor cell proliferation by 50 % (average values ± standard deviation).

pounds assayed for their inhibitory effects on tumor cell growth, ribavirin showed the greatest cytostatic potency, which appears conform to previously findings of Müller et al.[17]

Experimental section

Chemistry. Melting points were determined in open capillaries on a Büchi SMP-20 apparatus and are uncorrected. Proton magnetic resonance (^{1}H NMR) spectra were obtained with a Jeol FX-90Q spectrometer in dimethyl-d_6 sulfoxide. The chemical shift values are expressed in δ, parts per million, relative to tetramethylsilane as an internal standard. Ultraviolet (UV) spectra were recorded in ethanol in a Zeiss Specord UV-Vis spectro-

photometer. Mass spectra were obtained on a Jeol JMS-D-100 instrument. Thin-layer chromatography (TLC) was conducted on Merck precoated silica gel F_{254} plates (0.25 mm). For a preparative short column chromatography Merck TLC silica gel type 60 H was used (100 g of adsorbent to 1 g of mixture, unless otherwise stated).

Evaporations were performed under reduced pressure below 40°C with a rotary evaporator. Elemental analyses were performed by the Microanalytical Laboratory, Institute of Organic Chemistry, Polish Academy of Sciences, Warsaw. All 5-R', 5-acylamino-6-amino-4,5-dihydropyrimidin-4-ones (4a-e) were prepared by condensation reactions of appropriate cyanoesters either with thiourea or with guanidinium carbonate in anhydrous ethanol in the presence of sodium ethoxide, essentially as described previously[11,12].

8-Allyl-6-methyl-2(1H)-thioxoimidazo[1,5-a]-1,3,5-triazin-4(3H)-one (5a). To a suspension of 5-acetylamino-5-allyl-6-amino-4,5-dihydro-2(3H)-thioxopyrimidin-4-one[18] (4a, 480 mg, 2.0 mmol) in anhydrous pyridine (7 mL) was added chlorotrimethylsilane (0.51 mL, 4 mmol) and the mixture was stirred at room temperature for 45 min. Hexamethyldisilazane (1.63 mL, 10.8 mmol) was then added and the mixture was refluxed under argon for 3 h. Examination by TLC in chloroform-methanol (4:1) showed that the reaction was complete (Rf values substrate 0.23, product 0.61). The solvents were removed in vacuo, the residue was stirred with absolute ethanol at 0°C for 10 min, treated with anhydrous ether and kept below 0°C for 2 h. The crystalline solid that separated out was collected by filtration, washed with cold water (2 mL) followed by anhydrous ether (10 mL) and dried to yield 0.373 g (84 %) of the chromatographically homogenous title compound.

Recrystallization from 2-butanone provided an analytical sample : mp 201°C dec; UV λ_{max} (EtOH) 260 nm (sh) (ε 11 154), 288 (14 230); ^{1}H NMR (Me_2SO-d_6) δ 2.55 (s, overlaps with Me_2SO-d_5, 6-CH_3), 3.32 (d,2,8-CH_2), 4.93-5.12 (m,2,CH=$\underline{CH}_2$), 5.66-6.10 (m,1,$\underline{HC}$=CH_2), 12.25 (br s,1,NH), 12.94 (br s,1, NH); MS m/z (rel. intensity 75 eV) 222 (M^+, 100), 181 (8), 180 (11), 179 (20), 178 (13), 94 (19). Anal. Calcd for $C_9H_{10}N_4OS$: C, 48.65; H, 4.50; N, 25.22. Found : C, 48.65; H, 4.37; N, 25.33

8-Allyl-6-methylimidazo[1,5-a]-1,3,5-triazin-4(3H)-one (5d). A solution of 5a (800 mg, 3.6 mmol) in hot ethanol (50 mL) was added dropwise to Raney nickel catalyst (3.5 g, deactivated by heating in boiling water for 6 h), and the mixture was heated under reflux for 4 h. TLC in solvent system acetone-benzene-25 % aq. ammonia (6:4:0.3, v/v) showed that under these conditions the desired product 5d (R_f 0.12) was not accompanied by a by-product, the 8-propyl derivative 5e (R_f 0.15). The mixture was filtered hot to remove the catalyst, which was then washed with hot ethanol (2 x 20 mL). The combined filtrate and washings were evaporated to dryness, the brown residue (786 mg) was dissolved in methanol and adsorbed on silica gel (5 g) by evaporation of the solvent. It was then applied onto a short silica gel column and eluted with ethyl acetate-2-propanol (99:1). Evaporation of the appropriate homogenous fractions gave 403 mg (59 %) of colorless, crystalline 5d: mp 169°C (recrystallized from 2-butanone; UV λ_{max} (EtOH) 263 nm (sh) (ε 8668), 267 (8990), 301 (sh) (4040); ^{1}H NMR (Me_2SO-d_6) δ 2.69 (s,3,6-CH_3), 3.37 (d,2,8-CH_2), 4.95-5.20 (m,2, CH=$\underline{CH_2}$), 5.77-6.21 (m,1,$\underline{CH}$=CH_2), 7.49 (s,1,2-H), 11.73 (br s,1,NH); MS m/z (rel. intensity, 75 eV) 190 (M^+, 100), 189 (24), 163 (17), 162 (12), 149 (13), 148 (15), 147 (27), 146 (17). Anal. Calcd for $C_9H_{10}N_4O$: C, 56.83; H, 5.30; N, 29.46. Found : C, 56.52; H, 5.26; N, 29.31.

6-Methyl-8-propylimidazo[1,5-a]-1,3,5-triazin-4(3H)-one (5e). Compounds 5e and 5d were formed in approximately equal amounts (50 % total) when 5a was subjected to desulfurization in aqueous ammonia according to [11]. The chromatographic mobility of 5e was identical with that of 5d in several TLC and column solvent systems. The separation of 5e was finally achieved on a TLC plate (0.5 mm), using acetone-benzene-25 % aq. ammonia (6:4:0.3) as a solvent. Chromatographically homogenous syrup, which failed to crystallize and to give exact analytical numbers. UV λ_{max} (EtOH) 263 nm (sh) (ε 3231), 269 (3310), 301 (sh) (1530); ^{1}H NMR ($CDCl_3$) δ 0.88 (t,3,$\underline{CH_3}CH_2CH_2$), 1.61 (sex,2,$CH_3\underline{CH_2}CH_2$), 2.66 (t,2,$CH_3CH_2\underline{CH_2}$), 2.79 (s,3,6-CH_3), 7.33 (s,1,2-H), 11.73 (br s,1,ex D_2O,NH); MS m/z (75 eV) 192 (M^+), 165, 149.

8-[(RS)-2,3-Dihydroxypropyl]-6-methylimidazo[1,5-a]-1,3,5-triazin-4(3H)-one (6a). Method A. To a solution of 5d (52 mg, 0.27 mmol) in anhydrous t-butanol (5 mL) were added catalytic amount of pyridine (0.3 mL)

and a 0.5 % solution of osmium tetroxide in t-butanol[19] (17 mL, 0.34 mmol OsO_4). The color of the solution became reddish-brown indicating the formation of the osmate ester-pyridine complex. After 6 h at room temperature the reaction was complete. The mixture was concentrated in vacuo to a volume of about 3 mL, treated with 40 % $NaHSO_3$ solution (0.3 mL) followed by 95 % ethanol (2.0 mL) and heated under reflux for 15 min. After cooling to 40°C the black solid that precipitated during reduction was separated by filtration through Celite. The filtrate was evaporated to dryness, the residue was dissolved in methanol (10 mL), silica gel (2.5 g) was added and the solvent was evaporated. It was then applied onto a short silica gel column and eluted with chloroform-methanol (9:1) followed by (4:1). The fractions containing the homogenous product were pooled and evaporated to give 6a as a white, crystalline solid (52 mg, 85 %): mp 186°C (recrystallized from hot ethanol); UV λ_{max} (EtOH) 262 nm (sh) (ε 8315), 268 (8539), 301 (sh) (3933); ^{1}H NMR (Me_2SO-d_6) δ 2.61-2.71 (s,5,6-CH_3 and 8-CH_2), 3.25 (d,2,$\underline{CH_2}OH$), 3.67-3.87 (m,1,$\underline{CH}OH$), 5.80 (br s,2,ex D_2O, 2 x OH), 7.48 (s,1,2-H); MS m/z (rel. intensity, 75 eV) 224 (M^+, 31), 194 (28), 193 (88), 164 (92), 163 (65), 136 (93), 121 (36), 43 (100). Anal. Calcd for $C_9H_{12}N_4O_3$: C, 48.21; H, 5.39; N, 24.99. Found : C, 48.00; H, 5.43; N, 24.67.

Method B. The reaction was performed in the same manner as in A using 5d (114 mg, 0.6 mmol) in t-butanol (15 mL), pyridine (2 mL) and osmium tetroxide (30 mL of 0.5 % solution in t-butanol, 0.6 mmol). The mixture was then treated upon stirring with thioacetamide (54 mg, 0.72 mmol) in water (0.5 mL). Stirring was continued at 40°C for 3 h. The solvents were then evaporated in vacuo, the residue was dissolved in hot methanol, filtered through Celite and adsorbed on silica gel by evaporation of the solvent. It was then chromatographed on a short silica gel column with a gradient of chloroform-methanol (10-20 % of methanol) to give 80 mg of still contaminated product. Further purification by preparative TLC followed by recrystallization from ethanol gave pure 6a (59 mg, 44 %).

Method C. To a stirred solution of 5d (245 mg, 1.3 mmol) in methanol (5 mL) were added sodium chlorate (816 mg, 7.7 mmol) in water (10 mL) and a 0.5 % solution of osmium tetroxide in t-butanol (5.5 mL, 0.11 mmol). After 44 h at room temperature an additional portion of osmium tetroxide solution was added (5.5 mL) and the reaction was continued for another 22 h. The solution was evaporated to dryness and the residue was purified on

a short silica gel column using chloroform-ethanol (4:1) as the eluant to give chromatographically homogenous 6a (232 mg, 80 %).

Attempted hydroxylation of 8-allyl-2-amino-6-methylimidazo[1,5-a]-1,3,5triazin-4(3H)-one (5c). A solution of 5c (700 mg, 3.39 mmol, prepared according to [12]) in dimethylformamide (30 mL) and osmium tetroxide (1 g, 3.39 mmol) in anhydrous pyridine (10 mL) was incubated at room temperature for 2 h. TLC in solvent system chloroform-methanol (4:1) showed complete disappearance of the substrate. The mixture was divided into two equal parts (approx. 20 mL, 1.7 mmol of expected product) which were separately evaporated to dryness and subjected to different osmate ester cleavage procedures. The first sample was stirred with $NaHSO_3$ (900 mg) in water (15 mL) and pyridine (17.5 mL) at room temperature for 5 h. The product decomposed into a UV transparent material. The second sample was dissolved in water (150 mL) and hydrogen sulfide was passed into the solution. The product identified in MS as 6b, MS m/z (15 eV) 239 (M^+), precipitated together with osmium dioxide and could be isolated in traces.

N-2-Acetylamino-8-allylimidazo[1,5-a]-1,3,5-triazin-4(3H)-one (5f). 8-Allyl-2-aminoimidazo[1,5-a]-1,3,5-triazin-4(3H)-one[12] (5b, 85 mg, 0.4 mmol) was heated under reflux with acetic anhydride (8 mL) for 30 min. The resulting clear solution was evaporated in vacuo, traces of acetic anhydride were removed by coevaporation with methanol and the residual solid (128 mg) was purified on a short silica gel column using chloroform-2-propanol (95:5) as a solvent. 5f crystallized out after concentration of the appropriate, pooled fractions to approx. 10 mL (66 mg, 63 %). An analytical sample was recrystallized from methanol-tetrahydrofuran (1:1): mp. 218°C dec.; UV λ_{max} (EtOH) 283 nm (ε 13 190); ^1H NMR (Me_2SO-d_6) δ 2.15 (s,3,$NHCOCH_3$), 3.37 (d,2, CH_2), 4.92-5.14 (m,2,$CH=CH_2$), 5.76-6.13 (m,1, $CH=CH_2$), 8.19 (s,1,6-H), 11.60 (br s,2,ex D_2O, 2 x NH); MS m/z (rel. intensity, 75 eV) 223 (M^+, 75), 191 (100), 190 (17), 164 (13), 43 (33). Anal. Calcd for $C_{10}H_{11}N_5O_2$: C, 51.50; H, 4.75; N, 30.03. Found : C, 51.41; H, 4.77; N, 30.09.

N-2-Acetylamino-8-allyl-6-methylimidazo[1,5-a]-1,3,5-triazin-4(3H)-one (5g). 8-Allyl-2-amino-6-methylimidazo[1,5-a]-1,3,5-triazin-4(3H)-one[12] (5c, 137 mg, 0.7 mmol) was stirred with acetic anhydride (6.9 mL)

at 90° for 1.5 h. The resulting solution was worked-up in the same manner as for 5f to give 211 mg of a solid. Purification on a short silica gel column using chloroform-2-propanol (9:1) as the solvent yielded 55 mg (33 %) of 5g which crystallized out after concentration of the appropriate combined fractions to approx. 5 mL. An analytical sample was recrystallized from methanol-tetrahydrofuran (2:1); mp 220°C dec.; UV λ_{max} (EtOH) 286 nm (ε 12 580); ^{1}H NMR (Me_2SO-d_6) δ 2.13 (s,3,NHCO$\underline{CH}_3$), 2.62 (s,3,6-CH_3), 3.29 (d,2,8-CH_2), 4.92-5.10 (m,2,CH=$\underline{CH}_2$), 5.71-6.08 (m,1,$\underline{CH}$=CH_2), 11.60 (br s,2,ex D_2O, 2 x NH); MS m/z (rel. intensity, 75 eV) 247 (M^+, 81), 205 (100), 204 (18), 164 (24), 163 (49), 43 (28). Anal. Calcd for $C_{11}H_{13}N_5O_2$: C, 53.43; H, 5.30; N, 28.32. Found : C, 53.54; H, 5.16; N, 28.08.

N-2-Acetylamino-8-[(RS)-2,3-dihydroxypropyl]imidazo[1,5-a]-1,3,5-triazin-4(3H)-one (6c). Methods A and C (cf preparation of 6a) were not applicable due to degradation taking place in the presence of $NaHSO_3$ (A) and $NaClO_3$ (C).

Method B. Compound 5f (140 mg, 0.6 mmol) was dissolved in pyridine (2 mL) and t-butanol (35 mL) at 40°C. To this solution a 0.5 % solution of osmium tetroxide in t-butanol (30 mL, 0.6 mmol OsO_4) was added and the mixture was stirred at room temperature for 3 h. Thioacetamide (160 mg, 2.1 mmol) in water (4 mL) was added and the stirring was continued at 45°C for 3 h. To isolate the desired 6c in pure form the work-up procedure was performed in two stages : (a) O-acetylation, (b) O-deblocking.

(a) N-2-Acetylamino-8-[(RS)-2,3-diacetyloxypropyl]imidazo[1,5-a]-1,3,5-triazin-4(3H)-one (6e). The mixture after the reduction of osmate ester was evaporated to dryness, the residue was treated with acetic anhydride (14 mL) and heated under reflux for 45 min. Acetic anhydride was removed in vacuo (the last traces by coevaporation with methanol) to leave a solid. It was triturated with chloroform (30 mL), the insoluble inorganic salts were filtered off and the filtrate was evaporated to dryness. The residue (231 mg) was dissolved in chloroform-ethanol (50:1) and separated on a short silica gel column using doubled portion of adsorbent (200:1) and chloroform-ethanol (50:1) as the eluant. The appropriate fractions combined and evaporated furnished crystalline, chromatographically homogenous 6e (151 mg, 72 %) : mp 196-201° (recrystallized from anhydrous 2-propanol); UV λ_{max} (EtOH) 282 nm (ε 13 152); ^{1}H NMR (Me_2SO-d_6) δ 1.96

(s,3,OCO$\underline{CH_3}$), 2.01 (s,3,OCO$\underline{CH_3}$), 2.16 (s,3, NHCO$\underline{CH_3}$), 2.91 (d,2,8-$\underline{CH_2}$), 4.08-4.33 (m,2,$\underline{CH_2}$OCOCH$_3$), 5.19-5.30 (m,1,$\underline{CH}$OCOCH$_3$), 8.22 (s,1,6-H), 11.61 (br s, 1,ex D_2O,NH), 11.75 (br s, 1,ex D_2O,NH); MS m/z (rel. intensity, 75 eV) 351 (M^+, 13), 291 (57), 248 (22), 206 (35), 164 (44), 122 (15), 44 (100). Anal. Calcd for $C_{14}H_{17}N_5O_6$: C, 47.86; H, 4.88; N, 19.93. Found : C, 47.84; H, 4.85; N, 19.81.

(b) A solution of 6e (100 mg, 0.3 mmol) in anhydrous methanol (55 mL) was stirred with anhydrous potassium carbonate (200 mg) at room temperature for 1.5 h. It was then neutralized with Dowex H^+50W resin, the resin was quickly filtered off and washed with methanol. The filtrate and washings were evaporated to a dry residue (68 mg) which was recrystallized from hot 65 % ethanol (45 mL) to give chromatographically homogenous 6c. After drying in vacuo (100°, 0.01 mmHg) 66 mg (87 %, 63 % overall yield from 5f) : mp 218-219° dec.; UV λ_{max} (EtOH) 283 nm (ε 11 333); ^{1}H NMR (Me_2SO-d_6) δ 2.14 (s,3,NHCO$\underline{CH_3}$), 2.62-2.70 (m,2,8-$\underline{CH_2}$), 3.33 (d,2,$\underline{CH_2}$OH), 3.81 (br m,1,$\underline{CH}$OH), 4.49-4.68 (two br s,2,ex D_2O, 2 x OH), 8.20 (s,1,6-H), 11.68 (br s,2,ex D_2O, 2 x NH); MS (75 eV) no molecular ion. Anal. Calcd for $C_{10}H_{13}N_5O_4.0.75\ H_2O$: C, 42.78; H, 5.16; N, 24.95. Found : C, 42.93; H, 5.13; N, 24.68.

N-2-Acetylamino-8-[(RS)-2,3-dihydroxypropyl]-6-methylimidazo[1,5-a]-1,3,5-triazin-4(3H)-one (6d). Method A not applicable (cf remark on 6c).

Method B. Compound 5g (148 mg, 0.6 mmol) in pyridine (2 mL) and t-butanol (25 mL) was stirred with 0.5 % solution of osmium tetroxide in t-butanol (30 mL, 0.6 mmol OsO_4) at room temperature for 6 h. Thioacetamide (54 mg, 0.7 mmol) in water (10 mL) was added and the stirring was continued at 40°C for 6 h.

(a) N-2-Acetylamino-8-[(RS)-2,3-diacetyloxypropyl]-6-methylimidazo[1,5-a]-1,3,5-triazin-4(3H)-one (6f). The acetylation and chromatographic purification was performed by a procedure similar to the one used to prepare 6e. The material after short column chromatography (125 mg, 57 %) was still contaminated. Recrystallization from anhydrous 2-propanol furnished pure 6f as colorless crystals (44 mg, 26 %); mp 140-144°C. UV λ_{max} (EtOH) 283 nm (ε 13 295); ^{1}H NMR (Me_2SO-d_6) δ 1.97 (s,3,OCO$\underline{CH_3}$), 2.01 (s,3,OCO$\underline{CH_3}$), 2.14 (s,3,NHCO$\underline{CH_3}$), 2.62 (s,3,6-$\underline{CH_3}$), 2.81 (d,2,8-$\underline{CH_2}$), 4.06-4.34 (m,2,$\underline{CH_2}$OCOCH$_3$), 5.15-5.26 (m,1,$\underline{CH}$OCOCH$_3$), 11.48 (br s,2,ex D_2O, 2 x NH); MS m/z (rel. intensity, 75 eV) 365 (M^+, 13), 305 (55), 263

(18), 262 (100), 220 (61), 204 (13), 178 (66), 136 (35). Anal. Calcd for $C_{15}H_{19}N_5O_6$: C, 49.31; H, 5.24; N, 19.17. Found : C, 49.52; H, 5.24; N, 18.93.

(b) A solution of 6f (30 mg, 0.08 mmol) in anhydrous methanol (10 mL) was stirred with anhydrous potassium carbonate (25 mg) for 1 h. Following the work-up procedure analogous to that described for 6c, pure crystalline 6d crystallized out from the solution immediately after neutralization (22 mg, 96 %, 25 % overall yield from 5g) : mp 238-239°C dec (recrystallized from boiling 50 % ethanol, dried in vacuo at 100°C, 0.01 mmHg); UV λ_{max} 285 nm (ε 11 550); ^{1}H NMR (Me_2SO-d_6) δ 2.13 (s,3,NHCOC$\underline{H}_3$), 2.5 (8-CH_2, overlaps with $(Me)_2SO-d_5$), 2.63 (s,3,6-CH_3), 3.30 (br s, $\underline{CH}_2$-OH+H_2O), 3.73 (br m,1,$\underline{CH}$OH), 4.47 (br s,2,ex D_2O, 2 x OH), 11.45 (br s,2,ex D_2O, 2 x NH); MS m/z (rel. intensity 75 eV) 281 (M^+, 26), 250 (29), 221 (23), 220 (23), 179 (19), 178 (100), 136 (41), 135 (13). Anal. Calcd for $C_{11}H_{15}N_5O_4.0.75\ H_2O$: C, 46.22; H, 5.47; N, 24.51. Found : C, 46.26; H, 5.42; N, 24.22.

Method C. To a stirred solution of 5g (62 mg, 0.25 mmol) in tetrahydrofuran (6 mL) were added sodium chlorate (106 mg, 1 mmol) in water (6 mL) and a 0.5 % solution of osmium tetroxide in t-butanol (0.4 mL, 0.008 mmol OsO_4). Stirring was continued at room temperature for 24 h. The product gradually precipitated out as a colorless solid. Although after 24 h the substrate was still present in the solution (as indicated by TLC, Rf 0.68 in chloroform-methanol (4:1)) the reaction did not proceed further. The precipitate was collected by filtration and washed twice with tetrahydrofuran. After drying in vacuo (100°C, 0.01 mmHg) 44 mg (63 %) of chromatographically homogenous product was obtained.

8-Isopropyl-6-methyl-2(1H)-thioxoimidazo[1,5-a]-1,3,5-triazin-4(3H)-one (5h). 5-Acetylamino-6-amino-4,5-dihydro-5-isopropyl-2(3H)-thioxopyrimidin-4-one[18] (4d, 847 mg, 3.5 mmol) in anhydrous pyridine (12 mL) was treated with chlorotrimethylsilane (0.9 mL, 7 mmol) and hexamethyldisilazane (2.86 mL, 18.83 mmol) as described in the procedure for preparation of 5a. The reaction was complete after 2.5 h. The residue after the removal of solvents was quickly dissolved in warm, anhydrous ethanol (5 mL), immediately cooled to 0°C and treated with cold water (30 mL) causing the separation of a crystalline solid. Further portions crystallized out gradually to give 636 mg (81 %) of the title compound : mp 205°C; UV λ_{max}

(EtOH) 261 nm (sh) (ε 10 830), 287 (13 830); ^{1}H NMR (Me_2SO-d_6) δ 1.12 (d,6,$CH(\underline{CH_3})_2$), 2.56 (s, overlaps with Me_2SO-d_5,6-CH_3), 3.14-3.36 (m,1, $\underline{CH}(CH_3)_2$), 12.21 (br s,1,ex D_2O,NH), 12.91 (br s,1,ex D_2O,NH); MS m/z (rel. intensity, 75 eV) 224 (M^+, 100), 210 (13), 166 (12), 150 (84), 86 (14). Anal. Calcd for $C_9H_{12}N_4OS.0.25\ H_2O$: C, 47.23; H, 5.51; N, 24.50. Found : C, 47.33; H, 5.44; N, 24.73.

8-Benzyl-6-methyl-2(1H)-thioxoimidazo[1,5-a]-1,3,5-triazin-4(3H)-one (5i). 5-Acetylamino-6-amino-5-benzyl-4,5-dihydro-2(3H)-thioxopyrimidin-4-one[18] (4e, 1.392 g, 4.8 mmol) in anhydrous pyridine (16 mL) was treated with chlorotrimethylsilane (1.22 mL, 9.6 mmol) and hexamethyldisilazane (3.92 mL, 25 mmol) as described in the procedure for preparation of 5a. The reaction was complete after 2.2 h. The residue after removal of solvents was quickly dissolved in warm anhydrous ethanol (12 mL) immediately cooled to 0° and then kept at -10°C for 12 h. The bright-red precipitate that separated out was filtered off and recrystallized from anhydrous ethanol (10 mL) to give pure 5i (506 mg, 39 %). The remaining filtrates were combined and evaporated to dryness. The resulting residue (870 mg) was applied on a short silica gel column and eluted with chloroform-ethanol (95:5) followed by (9:1). The required fractions were pooled and evaporated to dryness to give a white foam, which after recrystallization from anhydrous ethanol (6 mL) provided an additional amount of product (493 mg, 38 %, making total yield 77 %). An analytical sample was recrystallized from anhydrous ethanol : mp 243-244°C dec; UV λ_{max} (EtOH) 261 nm (ε 11 130), 291 (14 900); ^{1}H NMR (Me_2SO-d_6) δ 2.51 (s, overlaps with Me_2SO-d_5,6-CH_3), 3.90 (s,2,8-CH_2 benzylic), 7.23 (s,5, aromatic), 12.19 (br s,1, ex D_2O,N-1 H), 13.17 (br s,1, ex D_2O,N-3 H); MS m/z (rel. intensity, 75 eV) 272 (M^+, 100), 230 (22), 229 (24), 171 (17), 117 (8), 91 (15). Anal. Calcd for $C_{13}H_{12}N_4OS$: C, 57.34; H, 4.44; N, 20.57. Found : C, 57.29; H, 4.27; N, 20.39.

Biological activity. The detailed procedures for measuring antiviral activity and antitumor cell activity are described in references 20 and 21, respectively.

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