NEW SYNTHETIC S-ADENOSYL-HOMOCYSTEINE ANALOGUES WITH ONCOSTATIC AND ANTIVIRAL ACTIVITY

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Description of the synthesis of 16 new nucleosides and their activity as inhibitors of transformation of chick embryo fibroblasts by Rous Sarcoma Virus and, in vitro, of tRNA methylases and protein methylase I ; the relation between chemical structure and biological activity is discussed.

S-adenosyl-L-homocysteine (SAH) being a strong inhibitor of all S-adenosyl-L-methionine (SAM) dependent transmethylases, several authors have studied the inhibitory activity of synthetic SAH analogues on more or less purified transmethylases in vitro [1-5]. A 7-deaza-analogue of SAH (S-tubercidinyl homocysteine) (STH) was shown recently to be an effective inhibitor of tRNA methylation in cultured, stimulated rat lymphocytes[6] and of dopamine methylation in murine neuroblastoma cells[7]. More recently STH, which is not degraded by enzymes responsible for SAH metabolism, was also shown to inhibit the methylation of m-RNA in Novikoff hepatoma cells ; it inhibits methylation at several sites (base methylation as well as 2'-O-methylation[8]).

In previous publications we have reported some interesting biological activities of synthetic analogues of S-adenosyl-L-homocysteine (SAH) in cell culture.

Abbreviations : CEF : chick embryo fibroblasts ; RSV : Rous Sarcoma Virus ; SAH : S-adenosyl-homocysteine ; SAM : S-adenosyl-methionine ; SIBA : 5'-deoxy-5'-S-isobutyl-thioadenosine ; STH : S-tubercidinyl homocysteine.

The most largely studied SAH analogue was 5'-deoxy-5'-S-isobutyl thioadenosine (SIBA) (1)[1] which strongly inhibits oncogenic transformation of chick embryo fibroblasts (CEF) by Rous Sarcoma virus (RSV)[9], polyoma virus replication in mouse embryo fibroblasts[10], the replication of Herpes virus[11], of mouse mammary tumor virus[12], mitogen induced blastogenesis of human and rabbit lymphocytes[13] and the multiplication of the malaria parasite (Plasmodium falciparum) in erythrocyte cultures[14] ; the inhibitory activity of SIBA against Friend's virus in vitro and in vivo in mice has been studied by Dr J.C. Cherman, (Institut Pasteur, personal communication).

In a preceding paper[15] we described the synthesis of several new analogues of SAH, derivatives of 5'-deoxy-adenosine which had been screened for their activity of inhibiting oncogenic transformation of CEF by RSV. The following were found to be the most active, (in order of decreasing activity) :(Fig. 1).

1 $R_1 = H$; $R_2 = SCH_2CH(CH_3)_2$

1a $R_1 = H$; $R_2 = SCH_2SCH_3$

1b $R_1 = H$; $R_2 = SCH_2CH{=}CH_2$

1c $R_1 = H$; $R_2 = SCH_2(CH_3)CH_2CH_3$

1d $R_1 = Bz$; $R_2 = SCH_2CH(CH_3)_2$

1e $R_1 = H$; $R_2 = SCH_2CH(NH_2)COOH$ (D)

1f $R_1 = H$; $R_2 = SCH_2CHOHCH_2OH$

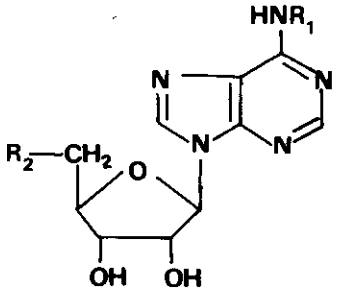
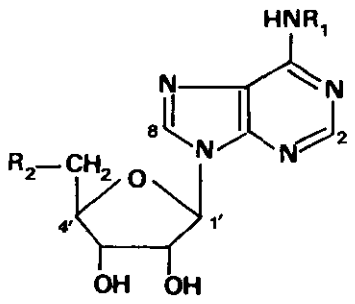
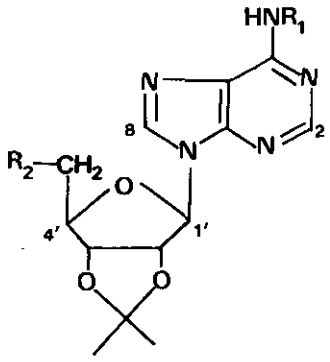


fig.1

In view of obtaining more active, more water soluble and metabolically more stable compounds, we have continued to prepare new analogues and describe now 16 new derivatives of which several are as active as SIBA against the oncogenic Rous sarcoma virus.





1 R_1 = H ; R_2 = $SCH_2CH(CH_3)_2$

2 R_1 = H ; R_2 = $SCH_2C(CH_3)=CH_2$

3 R_1 = H ; R_2 = $SCH_2CHOHCH_3$

4 R_1 = H ; R_2 = β-S-Glucose

6 R_1 = Me ; R_2 = $SCH_2CH(CH_3)_2$

8 R_1 = Me ; R_2 = $SCH(CH_3)CH_2CH_3$

9 R_1 = CH_2COOEt ; R_2 = OH

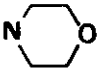
13 R_1 = CH_2COOMe ; R_2 = $SCH_2CH(CH_3)_2$

14 R_1 = CH_2COOH ; R_2 = $SCH_2CH(CH_3)_2$

16 R_1 = CH_2COOMe ; R_2 = $SCH_2CH=CH_2$

17 R_1 = CH_2COOH ; R_2 = $SCH_2CH=CH_2$

18 R_1 = H ; R_2 = $N(CH_3)CH(CH_3)_2$

19 R_1 = H ; R_2 = 

5 R_1 = Me ; R_2 = $SCH_2CH(CH_3)_2$

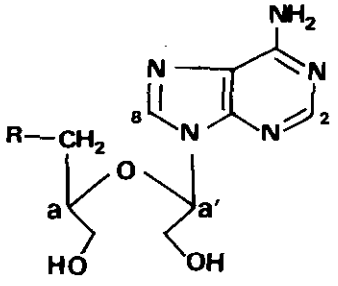
7 R_1 = Me ; R_2 = $SCH(CH_3)CH_2CH_3$

10 R_1 = CH_2COOEt ; R_2 = OH

11 R_1 = CH_2COOEt ; R_2 = O-tosyl

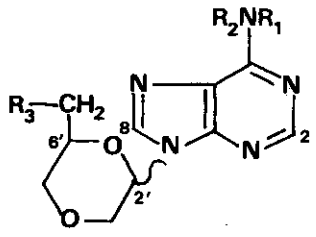
12 R_1 = CH_2COOMe ; R_2 = $SCH_2CH(CH_3)_2$

15 R_1 = CH_2COOMe ; R_2 = $SCH_2CH=CH_2$



20 R = Cl

21 R = $SCH_2CH(CH_3)_2$

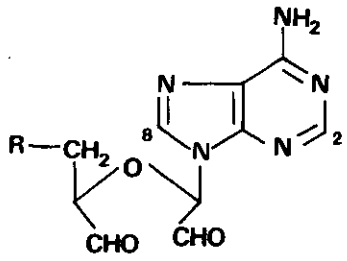


22 $R_1=R_2=H$; $R_3=OH$

23 $R_1=R_2=Me$; $R_3=OMe$

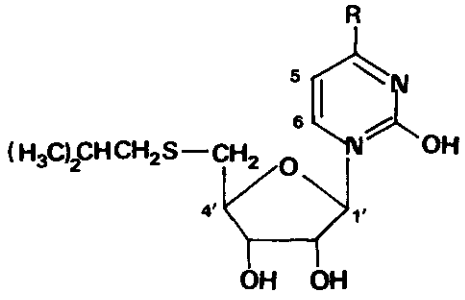
24 $R_1=R_2=H$; $R_3=$ O-tosyl

25 $R_1=R_2=H$; $R_3=SCH_2CH(CH_3)_2$



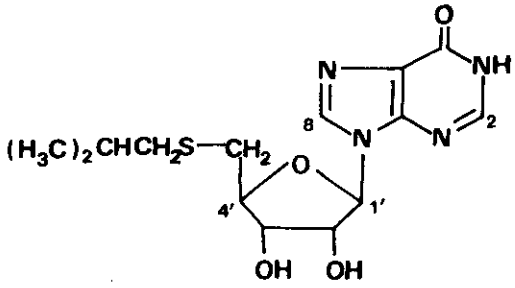
26 $R=SCH_2CH(CH_3)_2$

27 $R=S(=O)CH_2CH(CH_3)_2$



28 $R=NH_2$

29 $R=OH$



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The following modifications of the SAH-SIBA structure are described in this paper :

1 - replacement of the isobutyl radical of SIBA by allyl (17) methylallyl (2), hydroxypropyl (3) or D-glucose (4).
2 -replacement of 6 NH_2 of adenine by $NHCH_3$ (6,8) or $NHCH_2COOH$ (14-17).
3 - modification of the ribose moiety (21-27).
4 - replacement of the S-isobutyl moiety by a N-methyl isopropyl (18) or a morpholino group (19).
5 - replacement of adenine by cytosine (28) uracile (29) and hypoxanthine (30).

The most active molecules in the CEF-RSV test were compounds 2, 14, 17 and 27, whereas 21, 28 and 29 were inactive.

EXPERIMENTAL*

All melting points were taken on a Reichert melting point microscope and are uncorrected. The UV spectra were recorded on a Baush and Lomb spectronic 505 spectrometer. Mass spectra were determined on a MS-50 AEI spectrometer (Electron impact) or a MS-9 AEI spectrometer (Chemical ionisation) ; $[\alpha]_D$ were taken on a Jouan and Roussel polarimeter; IR spectra were recorded on a Perkin Elmer 257 spectrometer, pmr spectra were determined on a Varian T60 or Varian EM 360 spectrometers, in deuterated solvents using TMS as internal standard (δ TMS = 0 ppm).

Elemental analyses were determined by the Laboratoire de Microanalyse du CNRS, Gif sur Yvette, France. Thin layer chromatography was performed on Schleicher and Schüll F1500-LS254 silica gel plates.

* Abbreviations : tlc : thin layer chromatography ; bp : boiling point ; mp : melting point ; ms : mass spectrum ; pmr : proton magnetic resonance ; s : singlet ; d : doublet ; t : triplet , q : quintuplet ; m : multiplet ; b : broad.

5'-deoxy-5'-S-(2-methylallyl)-5'-thioadenosine (2)

To a solution of 1g (3.5 mmoles) of 5'-chloro-5'-deoxyadenosine in 15 ml of dimethylformamide are added at 0°, under nitrogen and with magnetic stirring, 120 mg of sodium hydride and 460 mg (5.2 mmoles) of 2-methylallyl mercaptan (Parish, redistilled bp : 92-93°C). The reaction mixture is kept 3 hrs at room temperature, then neutralized with HCl. The solvent is evaporated in vacuo (0.1 mm). The residue is dissolved in 50 % aqueous ethanol and poured on to a column of 25g Amberlite IR 120 H^+ ; the column is washed with the same solvent to neutrality and the compound eluted with an aqueous alcoholic solution of 2 N ammonia. After evaporation of the solvent the compound is recrystallized in ethanol-1 propanol 1:1, v/v). mp : 85-87°C ; yield 70 % (calcd. on 5'-chloro-5'-deoxyadenosine), Rf : 0.35 (EtOAc : MeOH, 8:2) ; pmr (DMSO;δ TMS = 0 ppm) δ : 1.8 (3H, s, CH_3), 2.6 to 3.30 (4H, m, $2CH_2$), 4.2 (3H, m, H_3,H_4, and $=CH_2$), 4.9 (2H, b.s., H_2, and $=CH_2$), 5.5 (2H, m, 2OH), 6 (1H, d (J = 5 Hz) H_1, β), 7.4 (2H, s, NH_2), 8.4 and 8.5 (2H, 2s, H_2 and H_8); ms, (Electron impact):m/e = 337 ($M^{+\cdot}$) ; m/e = 164 (B + 30) ; m/e = 136 (B + 2H).

Anal. Calcd for $C_{14}H_{19}O_3N_5S$, 1/2 H_2O : C, 48.53 ; H, 5.81 ; O, 16.16 ; N, 20.21 ; S, 9.25. Found : C, 48.10 ; H, 5.76 ; O, 16.36 ; N, 20.40 ; S, 9.41.

5'-deoxy-5'-S-(2-hydroxypropyl)-5'-thioadenosine (3)

1g (3.5 mmoles) of 5'-chloro-5'-deoxyadenosine is dissolved in 8 ml of 2N aqueous NaOH by warming ; then 0.65g (7 mmoles) of 2-hydroxypropanethiol (1-mercapto-2-propanol, Parish, bp 51°/12 mm) is added. After heating for 90 min. to 80°C, the solution is acidified with acetic acid to pH 2-3 and then evaporated to dryness. The compound is recrystallized in propanol and ethanol. Yield 75 %, mp 108-110°C, Rf : 0.32 (EtOAc : MeOH, 7:3) ; pmr (DMSO;δ TMS = 0 ppm) δ : 1.1 (3H, d, CH_3), 2.5 (2H, d, CH_2S), 2.9 (2H, d, H_5,), 3.1 (1H, s, OH), 3.7 (1H, q, CH), 4.1 (2H, m, H_3, and H_4,), 4.7 (1H, t, H_2,), 5.35 (2H, broad m, 2OH), 5.9 (1H, d, J = 5 Hz, H_1,, β), 7 (2H, broad s, NH_2), 8.10 and 8.3 (2H, 2s, H_2 and H_8); ms, (Electron Impact) : m/e = 341 ($M^{+\cdot}$) ; m/e = 323

($M^{+\cdot}$-18) ; m/e = 250 ($M^{+\cdot}$-91) ; m/e = 164 (B + 30) ; m/e = 136 (B + 2H).

Anal. Calcd for $C_{13}H_{19}O_4N_5S$, 1/2 H_2O : C, 44.55 ; H, 5.75 ; O, 20.54 ; N, 19.98 ; S, 9.15. Found : C, 44.53 ; H, 5.85; O, 20.85 ; N, 20.14 ; S, 9.18.

5'-deoxy-5'-S(β-D-glucopyranosyl)-5'-thioadenosine (4)

This compound was prepared by a modified Hutson method[16]: to 360 mg (1 mmol) of commercial tetracetyl-β-D-thioglucose (Aldrich) in 20 ml of dimethylformamide, 1 equivalent of 5'-O-tosyl-adenosine and freshly prepared sodium methoxide were added. After 5 hrs shaking a small quantity of sodium methoxide was added to complete the reaction. After neutralisation by a few drops of acetic acid and evaporation of the solvent, the oil thus obtained was peracetylated with a mixture of acetic anhydride : pyridine (1:1, v/v). The nucleoside was then extracted with chloroform, the organic phase was neutralized, washed and then dried.

TLC showed the presence of one product (Rf : 0.3 in ethyl acetate). This was deacetylated in anhydrous methanol in presence of sodium methoxide (yield 80 % calculated on thioglucose).

The free nucleoside 4 is then purified by paper chromatography (isopropanol) : H_2O, 65:35). Amorphous solid ; yield 50 % ; Rf : 0.6 (paper ; isopropanol : H_2O, 65:35) ; λmax : 260 nm (H_2O) $[\alpha]_D^{20°}$ = -40° (c = 0.6 ; MeOH) ; pmr (DMSO + D_2O ; δ TMS = 0) δ : 2.9 to 3.4 (m, $H_{2''}$ to $H_{6''}$ and $H_{5'}$), 4.2 (m, $H_{1''}$ $H_{3'}$ and $H_{4'}$), 4.76 (t, $H_{2'}$), 5.93 (d, J = 5 Hz, $H_{1'}$(β)), 8.1 and 8.3 (2 s, H_2 and H_8) ; pmr (D_2O, δ TMS = 0) δ : 3 to 3.5 (m, 6H, $H_{2''}$ to $H_{6''}$), 3.8 (2H, d, $H_{5'}$), 4.5 (1H, d, J = 8 Hz, $H_{1''}$(β)), 6 (1H, d, J = 5 Hz, $H_{1'}$ (β)), 8.2 and 8.4 (2H, 2 s, H_2 and H_8 of adenine) ; ms , (Electron impact ; peracetate) : m/e = 739($M^{+\cdot}$) ; m/e = 680 ($M^{+\cdot}$-59) ; m/e = 620 ($M^{+\cdot}$-119) ; m/e = 408 ($M^{+\cdot}$-331) ; m/e = 331 (oxonium ion) ; m/e = 206 (B + 30) ; m/e = 178 (B + 2H).

5'-deoxy-5'-S-(isobutyl)-2',3'- O-isopropylidene-N^6-methyl-5'-thioadenosine (5)

0.5 ml (4.8 mmoles) of 2-methyl-1-propanethiol was poured in 40 ml of cooled liquid ammonia and sodium was added until the solution remained dark blue for 5 min.

1.8 g (3.9 mmoles) of N^6-methyl-5'-toluenesulfonyl-2',3'-O-isopropylideneadenosine (prepared via tosylation and methylation of 2',3'-isopropylideneadenosine [17]) was then added and the reaction was stirred at room temperature over 5 hrs. Residual ammonia was evaporated and the crude product was taken into water (200 ml) and chloroform (100 ml). The aqueous layer was extracted and the combined organic extracts were washed, dried and evaporated to dryness to leave a yellow viscous oil.

The product is purified by chromatography (ethyl acetate) and 1.06 g of pure 2',3'-isopropylidene-5'-thionucleoside was isolated as a pale yellow oil in 70 % yield.

Rf : 0.4 (EtOAc) : λmax = 266 nm (EtOH) ; $[\alpha]_D^{20°}$ = -20° (c=1, EtOH) ; pmr ($CDCl_3$ δ TMS = 0) δ : 0.9 (6H, 2s, CH_3), 1.4 and 1.6 (6H, 2s, CH_3), 1.7 (1H, m, CH), 2.4 (2H, d, CH_2S), 2.76 (2H, d, $H_{5'}$), 3.15 (3H, d, NMe), 4.33 (1H, m, $H_{4'}$), 5.03 (1H, dd, $H_{3'}$), 5.53 (1H, dd, $H_{2'}$), 6.03 (1H, d, $H_{1'}$), 7.8 and 8.3 (2H, 2s, H_2 and H_8 of adenine) ; ms (Electron impact) m/e = 393 ($M^{+\cdot}$) ; m/e = 378 ($M^{+\cdot}$-15) ; m/e = 336 ($M^{+\cdot}$-57) ; m/e = 304 ($M^{+\cdot}$-89) ; m/e = 245 (oxonium ion).

The 2',3'-isopropylidene group was then removed by dissolving the oil in a 70 % solution of formic acid ; the solution was left 16 hrs at room temperature, evaporated to dryness and the product 6 crystallized from aqueous methanol to give white crystals in 95 % yield.

5'-deoxy-5'-S-(isobutyl)-N^6-methyl-5'-thioadenosine (6):
mp 143-145°C ; λmax = 266 nm (EtOH) ; $[\alpha]_D^{20°}$ = 0° (c=0.9, EtOH) pmr (DMSO + D_2O;δ TMS = 0) δ; 0.87 (6H, 2s, CH_3), 1.66 (1H, m, CH), 2.4 (2H, d, CH_2S), 2.76 (2H, d, $H_{5'}$), 3.03 (3H, s, NMe), 4.10

(2H, m, $H_{3'}$ and $H_{4'}$), 4.76 (1H, m, $H_{2'}$), 6 (1H, d, J = 5 Hz, $H_{1''}$, β), 8.36 and 8.43 (2H, 2s, H_2 and H_8).

Anal. Calcd for $C_{15}H_{23}N_5O_3S$, 1/2 H_2O : C, 49.70; H, 6.67; N, 19.32 Found : C, 49.55; H, 6.54; N, 18.92

5'-deoxy-2',3'-O-isopropylidene-5'-S-(1-methyl,1-propyl)-N^6-methyl-5'-thioadenosine (7): The same procedure was used as for the previous preparation. Yield, 75 % ; Rf : 0.4 (EtOAc) : λmax = 266 nm (EtOH) $[\alpha]_D^{20}$ = -18 (c=1.9, EtOH) ; pmr ($CDCl_3$;δ TMS = 0) δ : 0.7 to 1.7 (8H, m, $2CH_3$ and CH_2), 2.8 (2H, d, $H_{5'}$), 3.2 (3H, d, NMe), 7.8 and 8.3 (2H, 2s, H_2 and H_8) ; ms (Electron impact), m/e = 393 ($M^{+\cdot}$) ; m/e = 378 ($M^{+\cdot}$-15) ; m/e = 336 ($M^{+\cdot}$-57) m/e = 149 (B + H).

5'-deoxy-5'-S-(1-methyl, 1-propyl)-N^6-methyl-5'-thioadenosine (8) : Yield : 95 % (calcd. on 7) ; mp : 141-143°C ; $[\alpha]_D^{20°}$ = 0 (c=0.3, EtOH) ; λmax = 266 nm (EtOH) ; pmr (DMSO + D_2O; δ TMS = 0) δ : 0.7 to 1.67 (8H, m, $2CH_3$ and CH_2), 2.7 (2H, d, $H_{5'}$), 3.06 (3H, s, NMe), 4.10 (3H, m, $H_{3'}$, $H_{4'}$ and CHS), 4.80 (1H, m, $H_{2'}$), 6.06 (1H, d, J = 5 Hz, $H_{1''}$, β), 8.36 and 8.43 (2H, 2s, H_2 and H_8).

Anal. Calcd for $C_{15}H_{23}N_5O_3S$, 1/2 H_2O : C, 49.70; H, 6.67 ; N, 19.32 Found : C, 49.25; H, 6.66; N, 18.85

Ethyl-N^6-carboxymethyl adenosine (9): 3.75g (13 mmoles) of 6-chloro-9-β-ribofuranosyl 9H-purine (prepared from inosine[18]) were stirred with 2g (14.5 mmoles) of ethyl glycine hydrochloride in 100 ml of 1-butanol and 15 ml of triethylamine at 100°C for 5 hrs. The solution was then evaporated to dryness the residue dissolved in water and extracted with chloroform, giving 3.8 g of yellow oil which crystallized in ethanol to give 3.5 g of white crystals.

mp 92-95°C ; yield, 77 % ; Rf. : 0.6 (EtOAc : EtOH, 1:1) ; λmax : 266 nm (EtOH) ; $[\alpha]_D^{20}$ = -63° (c=1, EtOH) ; ms, (Electron impact) : m/e = 353 ($M^{+\cdot}$), m/e = 264 ($M^{+\cdot}$-89), m/e = 250 (B + 30), m/e = 222 (B + 2H).

Ethyl-N^6-carboxymethyl-2',3'-O-isopropylidene adenosine (10) : 3.40 g of product 9 (9.6 mmoles) were dissolved in 15 ml of formic acid containing 6 ml of 2,2-dimethoxypropane. The solution was stirred for 2 hrs and evaporated to dryness. The residue was then dissolved in chloroform and the solution brought to pH 7 by addition of 2N ammonia.

After extraction and evaporation, the 2',3'-O-isopropylidene derivative (3.45 g) was chromatographed on silica gel (eluent : ethyl acetate).

Yellow oil ; yield, 85 % ; Rf : 0.3 (EtOAc) ; λmax : 265 nm (EtOH) ; $[\alpha]_D^{20}$ = -66.5° (c=1.5, EtOH) ; pmr ($CDCl_3$;δ TMS = 0) δ : 1.27 (3H, t, CH_3), 1.37 and 1.63 (6H, 2s, $2CH_3$), 3.80 (2H, m, $H_{5'}$) δ : 4.20 (2H, q, CH_2), 4.37 (2H, d, NCH_2), 4.43 (1H, m, $H_{4'}$), 4.93 to 5.3 (2H, m, $H_{2'}$ and $H_{3'}$), 5.80 (1H, d, $H_{1'}$), 7.73 and 8.2 (2H, 2s, H_2 and H_8) ; ms, (Electron impact) : m/e = 393 ($M^{+\cdot}$), m/e = 378 ($M^{+\cdot}$-15), m/e = 304 ($M^{+\cdot}$-89), m/e = 250 (B + 30), m/e = 222 (B + 2H).

Ethyl-N^6-carboxymethyl-2',3'-O-isopropylidene-5'-O-tosyladenosine (11): This compound was prepared in the usual manner from 10. Pale tan foam ; yield, 83 % ; Rf : 0.35 (EtOAc) ; $[\alpha]_D^{20}$ = +23° (c=1, EtOH) ; pmr ($CDCl_3$;δ TMS = 0) δ : 1.3 (3H, t, CH_3), 1.37 and 1.57 (6H, 2s, CH_3), 2.37 (3H, s, CH_3Ar), 4.10 to 4.63 (5H, m, $H_{4'}$, $H_{5'}$,NCH_2), 4.7 (2H, q, CH_2), 4.93 (1H, d, $H_{3'}$), 5.23 (1H, dd, $H_{2'}$), 5.93 (1H, d, $H_{1'}$), 6.87 to 7.60 (4H, aromatic protons), 7.63 and 8.07 (2H, 2s, H_2 and H_8).

Methyl-N^6-carboxymethyl-5'-deoxy-2',3'-O-isopropylidene-5'-S-(isobutyl)-5'-thioadenosine (12): Compound 12 was obtained by treating 1g of ethyl ester (11) in THF with sodium and 2 equivalents of 2-methyl-1-propanethiol at room temperature for 20 hrs. After neutralisation with glacial acetic acid, the solution was evaporated to a thick oil, taken into chloroform and washed with water.

1 g of a mixture of ethyl- and 2-methyl-1-propanethio-esters was isolated (Rf : 0.45 and 0.55, ethyl acetate) readily converted to the pure methyl ester by treatment with 3 mg of sodium in 25 ml of methanol for 1 hr followed by neutralisation.

Pale yellow oil ; yield, 65 % ; Rf : 0.47 (EtOAc) ; λmax : 267 nm (EtOH) ; $[\alpha]_D^{20} = -16$ (c=1.7, EtOH) ; pmr ($CDCl_3$; δ TMS = 0) δ : 0.9 (6H, d, $2CH_3$), 1.4 and 1.6 (6H, 2s, $2CH_3$), 1.2 to 2.2 (1H, m, CH), 2.33 (2H, d, CH_2S), 2.73 (2H, d, $H_{5'}$), 3.73 (3H, s, OMe), 4.37 (2H, d, NCH_2), 4.53 (1H, m, $H_{4'}$), 4.97 and 5.43 (2H, 2dd, $H_{3'}$ and $H_{2'}$), 6 (1H, d, $H_{1'}$), 7.8 and 8.27 (2H, 2s, H_2 and H_8).

Methyl-N^6-carboxymethyl-5'-deoxy-5'-S-(isobutyl)-5'-thio adenosine (13):The 2',3'-O-isopropylidene group was removed by treatment with 80% aqueous formic acid for 2 hrs and the resulting oil was chromatographed eluting with ethyl acetate. It crystallized from methanol in 67 % yield.

mp 72-74°C ; yield, 67 % ; Rf : 0.27 (EtOAc) ; λmax : 266 nm (EtOH) ; $[\alpha]_D^{20} = -7.5°$ (c=2, EtOH) ; pmr($(CD_3)CO$; δ TMS = 0) δ : 0.79 (6H, d, $2CH_3$), 1.37 to 2.07 (1H, m, CH), 2.47 (2H, d, CH_2S), 2.93 (2H, d, $H_{5'}$), 3.67 (3H, s, OMe), 4 to 4.67 (4H, m, $H_{3'}$, $H_{4'}$ and NCH_2), 4.83 (1H, t, $H_{2'}$), 6.03 (1H, d, J = 5 Hz, $H_{1'}$,β), 8.3 and 8.37 (2H, 2s, H_2 and H_8); ms, (Electron impact) : m/e = 411 ($M^{+\cdot}$), m/e = 322 ($M^{+\cdot}$-89) ; m/e = 236 (B + 30), m/e = 208 (B + 2H).

Anal.Calcd for : $C_{17}H_{25}N_5O_5S$: C, 49.62 ; H, 6.12 ; N, 17.02; O, 19.44 ; S, 7.79. Found : C, 49.65 ; H, 6.14 ; N, 16.96, O, 19, 39 ; S, 7.82.

N^6-carboxymethyl-5'-deoxy-5'-S-(isobutyl)-5'-thioadenosine (14) : 170 mg (0.4 mmoles) of the methyl ester 13 were stirred in 6 ml of methanol while 6 ml of 0.1 N barium hydroxide were added dropwise. The solution was stirred until the hydrolysis was complete (30 min.). The mixture was then neutralized by addition of solid CO_2 and the resultant white suspension boiled to remove dissolved CO_2, cooled and then filtered.

The aqueous solution is evaporated to dryness and the nucleoside is precipitated from hot water : methanol (1:1);mp charring above 150°C ; yield, 80 % ; Rf : 0.7 (nBuOH : AcOH : H_2O, 4:1:1) ; λmax : 267 nm (H_2O) ; $[\alpha]_D^{20}$ = -4° (c=1, H_2O) ; pmr (DMSO;δ TMS = O) δ : 0.87 (6H, d, $2CH_3$), 1.40 to 2 (1H, m, CH), 2.40 (2H, d, CH_2S), 2.83 (2H, d, $H_{5'}$), 3.7 to 4.33 (4H, m, H_3, H_4, and NCH_2), 4.7 (1H, m, $H_{2'}$), 5.9 (1H, d, J = 5 Hz , $H_{1'}$,β), 8.27 and 8.37 (2H, 2s, H_2 and H_8)

Methyl-5'-S-allyl-N^6-carboxymethyl-5'-deoxy-2',3'-O-isopropylidene-5'-thioadenosine (15) was prepared by the same procedure as described above for (14). 15 was obtained in 70 % yield ; Rf : 0.43 (EtOAc) ; λmax : 266 nm (EtOH) ; $[\alpha]_D^{20}$ = -3° (c=2,2, EtOH) ; pmr ($CDCl_3$, δ TMS = O) δ: 1.4 and 1.6 (6H, 2s, $2CH_3$), 2.73 (2H, d, $H_{5'}$), 3.07 (2H, d, CH_2S), 3.7 (3H, s, OMe), 4.33 (2H, d, NCH_2), 4.36 (1H, m, $H_{4'}$), 4.92 to 5.03 (3H, m, $H_{2'}$ and 2H from $=CH_2$), 5.27 to 5.90 (2H, m, H_3, and H from =CHR), 6 (1H, d, J = 5 Hz, $H_{1'}$), 7.97 and 8.47 (2H, 2s, H_2 and H_8).

Methyl-5'-S-allyl-N^6-carboxymethyl-5'-deoxy-5'-thioadenosine (16). Mp 67-69°C (methanol : water) ; yield, 51 % ; Rf : 0.67 (EtOAc : EtOH, 1:1) ; λmax : 265 nm (EtOH) ; $[\alpha]_D^{20}$ = +6.5° (c=1.1, EtOH) ; ms, (Electron impact) : m/e = 395 ($M^{+\cdot}$), m/e = 354 ($M^{+\cdot}$-41), m/e = 322 ($M^{+\cdot}$-89), m/e = 236 (B + 30), m/e = 208 (B + 2H).

Anal.Calcd for : $C_{16}H_{21}O_5N_5S$, 1/2 H_2O : C, 47.51 ; H, 5.48 ; O, 21.76 ; N, 17.32. Found : C, 47.45 ; H, 5.40 ; O, 21.99 ; N, 17.46.

5'-S-allyl-N^6-carboxymethyl-5'-deoxy-5'-thioadenosine (17) Mp 150°C (decomposition) ; yield, 83.6 % ; Rf : 0.71 (nBuOH : AcOH : H_2O, 4:1:1) ; λmax : 268 nm (EtOH) ; $[\alpha]_D^{20}$ = + 10° (c=1.5, H_2O); pmr (DMSO;δ TMS = O) δ: 2.8 (2H, d, $H_{5'}$), 3.13 (2H, d, CH_2S), 3.6 to 4.3 (4H, m, H_3, H_4, and NCH_2), 4.57 to 5.10 (3H, m, $H_{2'}$ and 2H from = CH_2), 5.23 to 5.77 (1H, m, H_{trans} = CHR), 5.87 (1H, d, J = 5 Hz, $H_{1'}$, β), 8.13 and 8.23 (2H, 2s, H_2 and H_8).

5'-deoxy-5'-N-(isopropyl)-5'-N-methyl amino adenosine (18)

1g (2.36 mmoles) of 5'-O-tosyl adenosine (Aldrich, mp : 151-153°C) is dissolved in 10 ml of methyl-isopropylamine under nitrogen and stored 10 days with magnetic stirring. Solvent is then evaporated under reduced pressure and residue is dissolved in water (free from CO_2) and filtered through Amberlite-IR 45 (OH) ; the solution is evaporated to dryness and the nucleoside 18 is crystallized in propanol (saturated with ammonia).

Mp : 105-108°C (hygroscopic) ; yield, 68 % ; Rf : 0.21 (chloroform : MeOH, 8:2) ; pmr (DMSO; δ TMS = 0) δ : 1.05 (6H, d, CH_3), 2.30 (3H, s, NCH_3), 2.75 (2H, d, $H_{5'}$), 4.10 (2H, m, $H_{3'}$ and $H_{4'}$), 4.60 (1H, m, $H_{2'}$), 5.90 (1H, d, J = 5 Hz, $H_{1'}\beta$), 8.1 and 8.3 (2H, 2s, H_2 and H_8); ms, (Electron impact) : m/e = 322 ($M^{+\cdot}$), m/e = 307 ($M^{+\cdot}$-15), m/e = 250 ($M^{+\cdot}$-72), m/e = 164 (B + 30), m/e = 136 (B + 2H).

5'-deoxy-5'-N-morpholinoadenosine (19)

1g (3.5 mmoles) 5'-deoxy-5'-chloroadenosine is dissolved in 15 ml morpholine (Aldrich, bp : 128°) and the solution kept under stirring in a N_2 atmosphere for 36 h. After evaporation under reduced pressure the residue is dissolved in a mixture of $CHCl_3$: 3-methyl-butanol (1:2, v:v) in which a current of dry NH_3 gas is bubbled. The precipitate of ammonium chloride is centrifuged off and the solution evaporated under reduced pressure. The product is redissolved in 3-methyl-butanol and kept at 5°. The amorphous hygroscopic precipitate is centrifuged off.

Yield, 72 % ; Rf : 0.24 (EtOH : H_2O, 9:1, v:v) ; pmr (D_2O, δ TMS = 0) δ : 3.15 (4H, t, CH_2), 3.35 (2H, d, $H_{5'}$), 3.80 (4H, t, CH_2), 4.15 (2H, m, $H_{3'}$ and $H_{4'}$), 4.65 (1H, t, $H_{2'}$), 5.90 (1H, d, J = 5 Hz, $H_{1'}\beta$), 7.95 and 8.03 (2H, 2s, H_2 and H_8); ms, (Chemical Ionisation, Isobutane) : m/e = 337 (MH^+), m/e = 202 (oxonium ion), m/e = 136 (B + 2H), m/e = 88 (morpholinium ion).

2-O-[1-(9-adenyl)-2-(hydroxy)ethyl]-3-chloro-3-deoxy glycerol (20)

Compound 20 was obtained as an amorphous solid from 5'-deoxy 5'-chloroadenosine by periodate oxidation followed by reduction with $NaBH_4$ in water [19,20].

Amorphous solid ; yield, 60 % ; Rf : 0.45 (EtOAc : MeOH 8:2); pmr (DMSO;δ TMS = 0) δ: 3.5 (5H, m, $2CH_2$ et Ha), 3.9 (2H, d, CH_2) 5 (2OH), 5.84 (1H, t, Ha', pseudo anomeric proton), 7.2 (2H, s, NH_2), δ : 8.2 and 8.3 (2H, 2s, H_2 and H_8 from adenine), ms (Electron impact) : m/e =289 and 287 ($M^{+}\cdot$, isotopic ratio 1/3 2/3), m/e = 252 ($M^{+}\cdot$-HCl), m/e = 164 (B + 30), m/e = 136 (B + 2H).

This compound dissolved in aqueous 2N NaOH solution and treated by methyl propane thiol led to 21, while replacement of water by an aprotic solvent (DMF) gave the dioxane derivative 22.

2-O-[1-(9-adenyl)-2-(hydroxy) ethyl]-3-deoxy-3-S-isobutyl-3-thio glycerol (21)

Compound 21 was prepared by dissolving 290 mg (1 mmole) of 20 in aqueous 2N NaOH (100 ml) and addition of 2 equivalents of 2-methyl-propane-1-thiol. After heating for 2 hrs at 80°C, the reaction mixture is cooled, neutralized by a few drops of acetic acid, and evaporated to dryness.

The residue is then dissolved in 100 ml of water and extracted by chloroform (3 x 100 ml). After the usual work up the nucleoside 21 is obtained as colourless crystals from methanol.

Mp : 153-155°C ; yield, 80 % (calcd on 20) ; Rf : 0.7 (EtOAc : MeOH, 8:2) ; λmax = 260 nm (EtOH, H_2O) ; $[\alpha]_D^{20}$ = +61° (c=0.7, MeOH) ; pmr (DMSO;δ TMS = 0) δ: 0.7 and 0.9 (6H, 2s, CH_3) 1.8 (1H, m, CH), 2.9 (2H, d, CH_2S), 3.4 (5H, m, $2CH_2$ et CHa), 3.8 (2H, d, CH_2S), 4.5 (2H, broad s, 2OH), 5.8 (1H, t, Ha' pseudo anomeric proton), 7.1 (2H, s, NH_2), 8 and 8.2 (2H, 2s, H_2 and H_8 of adenine), ms (Electron impact) : m/e = 341 ($M^{+}\cdot$), m/e = 284 ($M^{+}\cdot$-57), m/e = 252 ($M^{+}\cdot$-89), m/e = 164 (B + 30), m/e = 136 (B + 2H).

9-[1',4'-dioxan-6'-(hydroxy methyl)-2'-yl] adenine (22)

Compound 20 treated by a freshly prepared solution of MeONa in DMF at 80°C during 4 hrs leads, after the usual work up, to the pseudo nucleoside 22.

Amorphous solid ; yield, 90 % ; Rf : 0.3 (EtOAc : MeOH, 8:2) λmax : 265 nm (EtOH) ; pmr (DMSO ; δ TMS = 0) δ : 3.4 to 4.5 (7H, m, CH_2 and $H_{6'}$), 4.7 (1H, OH), 6 (1H, m, $H_{2'}$, Jmax = 10 Hz[21,22], 7.3 (2H, s, NH_2), 8.2 and 8.5 (2H, 2s, H_2 and H_8 of adenine). After addition of a few drops of D_2O signals corresponding to OH and NH_2 protons are exchanged) ; ms, (Chemical Ionisation, Isobutane) : m/e = 252 (MH^+), m/e = 136 (B + 2H) ; ms, (Electron impact) : m/e = 251 ($M^{+\cdot}$), m/e = 233 ($M^{+\cdot}$), m/e = 233 ($M^{+\cdot}$-18), m/e = 220 ($M^{+\cdot}$-31), m/e = 164 (B + 30), m/e = 136 (N + 2H).

This product was further characterized by its mass spectrum after permethylation [23] (CH_3I, NaH) in a mixture of DMF, THF, giving (23): ms, (Chemical Ionisation, Isobutane) : m/e = 294 (MH^+), m/e = 164 (B + 2H) ; ms (Electron impact) m/e = 293 ($M^{+\cdot}$), m/e = 262 ($M^{+\cdot}$-OMe), m/e = 192 (B + 30), m/e = 163 (B + H).

9-[1',4'-dioxan-6'-(O-tosyl methyl)-2-yl] adenine (24): Compound 22 is tosylated by the usual method (tosyl chloride in pyridine) giving 24.

Rf : 0.6 (EtOAc : MeOH, 8:2) ; yield, 80 % ; pmr ($CDCl_3$; δ TMS = 0) δ :2.4 (3H, s, CH_3), 3.6 to 4.4 (7H, m, CH_2 and $H_{6'}$), 6 (1H, m, $H_{2'}$, pseudo anomeric proton), 7.4 and 7.7 (4H, AB system, aromatic proton), 8.2 and 8.5 (2H, 2s, H_2 and H_8 of adenine); ms (Electron impact) : m/e = 405 ($M^{+\cdot}$), m/e = 270 ($M^{+\cdot}$-B), m/e = 234 ($M^{+\cdot}$-OTs), m/e = 135 (B + H), m/e = 91.

9-[1',4'-dioxan-6'-(isobutyl thiomethyl)-2'-yl] adenine (25):

400 mg (1 mmole) of the tosylated pseudonucleoside 24 are dissolved in 20 ml of DMF ; 1.5 equivalents of 2-methyl-propane-1-thiol and fresh MeONa are then added and the reaction mixture is allowed to stand 5 hrs at 80°C. After neutralisation and evaporation to dryness the thionucleoside crystallized in methanol.

mp : 119-120°C ; yield, 80 % (calcd on 24) ; Rf : 0.65 (Et OAc : MeOH, 8:2) ; pmr ($CDCl_3$;δ TMS = 0) δ :0.8 and 1 (6H, 2s, CH_3), 1.7 (1H, m, CH), 2.4 (2H, d, CH_2S), 2.6 (2H, d, CH_2S), 3.4 to 4.5 (5H, m, H_3, H_5, et $H_{6'}$), 6.1 (1H, m, $H_{1'}$, pseudo anomeric proton), 6.85 (2H, broad s, NH_2), 8.35 and 8.5 (2H, 2s, H_2 and H_8 of adenine) ; ms (Electron impact) : m/e = 323 ($M^{+\cdot}$), m/e = 234 ($M^{+\cdot}$-89) m/e = 189 (pseudo oxonium ion), m/e = 164 (B + 30), m/e = 136 (B + 2H) ; ms, (Chemical Ionisation, Isobutane) : m/e = 324 (MH^+), m/e = 189 (pseudo oxonium ion), m/e = 136 (B + 2H).

2-O- [formyl (9-adenyl) methyl]-3-deoxy-3-S-isobutyl-3-thio glyceraldehyde (26)

This compound was prepared by periodate oxidation [24] of SIBA (1) as follows : to a solution of 500 mg of SIBA in 2 l water is added, drop by drop, a solution of 0.9 equivalent of sodium metaperiodate ; the reaction is monitored by tlc. (EtOAc : MeOH, 8:2). After 24 hrs the reaction mixture is evaporated, the residue dissolved in the minimum volume of water and extracted with warm chloroform. After the usual work up the oxidized nucleoside is obtained as amorphous solid.

Yield, 40 % ; Rf : 0.75 (EtOAc : MeOH, 8:2) ; λmax 260 nm (H_2O) $[\alpha]_D^{20}$ = -12° (c=0.5, DMSO) ; pmr (DMSO + D_2O, δ TMS = 0) δ : 0.9 (6H, m, CH_3), 2 (1H, m, CH), 2.6 (2H, d, CH_2S), 3 (2H, broad s, CH_2), 5 and 5.6 (2H, m, Ha and Ha'), 8.2 and 8.4 (2H, 2s, H_2 and H_8 of adenine. ms (Chemical Ionisation, isobutane) : m/e = 338 (MH^+), m/e = 136 (B + 2H). The reduction of 26 by $NaBH_4$ gives compound 21.

2-O- [formyl (9-adenosyl) methyl]-3-deoxy-3-S-isobutyl-3-thioglyceraldehyde sulfoxide (27)

Compound 26 is oxidized with 1 equivalent of sodium metaperiodate in water ; after 24 hrs stirring, the solution is partly evaporated under vacuum. The precipitate obtained is dissolved in the minimum volume of water ; addition of methanol precipitates periodate. The filtrate is then evaporated to dryness.

Amorphous solid, yield, 40 % (calculated on 26), Rf : 0.2 (EtOAc : MeOH, 8:2) ; $[\alpha]_D^{20}$ = -9° (c=0.9 DMSO) ; pmr (DMSO + D_2O δ TMS = 0) : very close to 26. ir : 870 cm^{-1}, 1000 cm^{-1} (sulfoxide).

5'-deoxy-5'-S-(isobutyl)-5'-thiocytidine (28)

1g (4 mmoles) of 5'-deoxy-5'-chlorocytidine [25] is dissolved in an aqueous 2N NaOH solution : 2 equivalents of 2-methyl-1-propanethiol are then added. The mixture is stirred at 80°C for 4 hrs. The reaction is monitored by tlc (EtOAc : MeOH, 8:2). Two thionucleosides are isolated after neutralisation (5 ml of acetic acid) and evaporation to dryness.

The two compounds are chromatographed and characterized as the nucleoside 28 and its uracil homolog [26] 29.

Experiment monitored 24 hrs led to 29 in 100 % yield.

Mp : 198-200°C ; yield : 30 % ; Rf : 0.3 (EtOAc : MeOH 8:2) λmax : 273 nm (pH 14, EtOH/H_2O) ; pmr (CD_3OD ; δ TMS = 0) δ :0.9 and 1 (6H, 2s, CH_3), 0.9 and 1 (6H, 2s, CH_3), 1.8 (1H, m(q), CH), 2.5 and 2.9 (4H, 2d, CH_2S and H_5,), 4.1 (3H, m, H_2,H_3, and H_4,), 5.86 (1H, d, J = 5 Hz, H_1, β), 5.9 and 7.8 (2H, 2d, AB system, J = 8 Hz, H_5 and H_6); ms,(Electron impact) : m/e = 315 ($M^{+\cdot}$) ; m/e = 205 ($M^{+\cdot}$-B), m/e = 112 (B + 2H).

5'-deoxy-5-S-(isobutyl)-5'-thio uridine (29)

Mp : 128-130°C ; yield, 60 % ; Rf : 0.8 (EtOAc : MeOH, 8:2); λmax : 262 (pH 14 EtOH/H_2O) ; pmr (CD_3OD;δ TMS = 0) δ :0.9 1 (6H, 2s, CH_3), 1.7 (1H, m(q), CH), 2.5 and 2.8 (4H, 2d, CH_2S and H_5,), 4.1 (3H, m, H_2, H_3, et H_4,), 5.8 (1H, d, J = 5 Hz, H_1, β), 5.7 and 7:7 (2H, 2d, AB system, J = 8 Hz, H_5 and H_6); ms, (Electron impact) m/e = 316 ($M^{+\cdot}$), m/e = 227 ($M^{+\cdot}$-89), m/e = 113 (B + 2H).

5'-deoxy-5'-S-(isobutyl)-5'-thio inosine (30)

To a solution of 1g (3.5 mmoles) of 5'-deoxy-5'-chloro inosine in 25 ml of dimethylformamide are added, with magnetic stirring and under nitrogen 130 mg (5.4 mmoles) of sodium hydride and 2 equivalents of 2-methyl-1-propanethiol (7 mmoles).

After 3 hrs the reaction mixture is evaporated and the residue is dissolved in aqueous acetic acid (10%) and poured on to a column of 25 g Amberlite IR 120 H^+. The column is washed with water to neutrality (500 ml) and the product eluted with a solution of 2N ammonia. After evaporation compound 30 is crystallized from a mixture of isoamyl alcohol-ammonia.

Mp : 179-181°C ; yield, 72 % ; Rf : 0.54 (EtOAc : MeOH, 7:3); pmr (DMSO, δ TMS = 0) δ :0.9 (6H, d, CH_3), 2.4 (2H, d, CH_2S), 2.85 (2H, d, $H_{5'}$), 4.10 (2H, m, $H_{3'}$ and $H_{4'}$), 4.65 (1H, t, $H_{2'}$), 5.85 (1H, d, J = 5 Hz, $H_{1'}$,β), 8.05 and 8.25 (2H, 2s, H_2 and H_8); ms, (Chemical Ionisation, Isobutane) : m/e = 341 (MH^+), m/e = 205 (oxonium ion), m/e = 137 (B + 2H).

Anal. Calcd for $C_{14}H_{20}N_4O_4S$,1/2 H_2O : C 48.12 ; H, 6.05 ; O, 20.60 ; S, 9.17. Found : C, 48.01 ; H, 5.96 ; O, 20.30 ; S, 9.53.

BIOLOGICAL ASSAYS

Effect of the analogues on normal cell multiplication

Secondary cultures of chick embryo fibroblasts were seeded at 5 x 10^5 cells/dish and 24 hrs later the analogues were added at the desired concentration. The cultures (in duplicate) were incubated at 37°. The cells from the control cultures and from the treated cultures were counted every 24 hrs.

To distinguish between cystostatic and cytotoxic effect, the inhibitor containing media were replaced by the standard growth medium after various incubation times and cells were counted again, one and two days later. A product was considered as only cytostatic if upon medium renewal 24 and 48 hrs later the cell number doubled.

Inhibition of cell transformation

Secondary CEF cultures were plated as before, and infected one day later with 100 FFU* of RSV. Cultures were then overlaid with 0.8 % Difco agar. Cytostatic concentrations (for normal cells) of the inhibitors in 1.5 ml of liquid medium were added on top of the gelled underlayer, immediately after virus adsorption. Control cultures were overlaid with inhibitor-free medium. After 2 days of exposure, the inhibitor containing liquid overlayer was replaced by the standard growth medium. Foci of transformed cells were counted 8 days later and their number compared with the number of foci from control cultures.

The preparation of the cell-free extracts and the determination of the tRNA methylase activity was the same, as described in a previous publication [9]. The protein methylase activities were measured as indicated by Paik et al.[33]. The concentration of SAM was always 10^{-4}M in the assays.

* Focus Forming Units

TABLE 1

Effect of synthetic SAH analogues on cell division of normal cells and on the oncogenic cell transformation induced by Rous sarcoma virus

Compound	Conc. producing reversible cytostatic effect on normal cells mM	% inhibition of cell transformation by the same concentration.
SAH	1.0	20
1 (SIBA)	0.50	100
	0.10	65
2	0.50	100
	0.10	35
3	0.50	87
4	0.50	80
6	0.50	39
8	0.50	59
14	0.50	100
17	0.50	100
18	0.50	24
19	0.50	82
21	0.50	0
25	0.50	40
26	0.50	53
27	0.25	90
28	0.50	0
29	0.50	0
30	1.0	25

TABLE 2

Effect of synthetic SAH analogues on tRNA and protein methylases of normal chick embryo fibroblasts *in vitro*.

Compound	Ki tRNA methylases μM	Ki Protein methylase I μM
SAH	4.6	1.5
1 (SIBA)	2190	182
2	1500	600
3	2724	794
4	∞	∞
6	513	2770
8	500	1660
14	1543	6000
17	1073	∞
21	∞	∞
25	∞	∞
26	∞	∞
27	700	471
28	580	∞
29	1120	∞
30	670	∞

Concentration of SAM : 10^{-4}M.

RESULTS AND DISCUSSION

This paper describes the synthesis of 16 new nucleosides, mostly adenine derivatives, and their activity as inhibitors of cell transformation induced by RSV, in cell culture, as well as their effect in vitro on tRNA methylases and protein methylase I (S-adenosyl-L-methionine : protein(arginine)N-methyltransferase, EC 2.1.1.23).

As shown in Table 1 the compounds having comparable biological activity to SIBA (1), which has been extensively studied previously [6-15] are : 2, 14, 17 and 27.

We already know [9, 15] that the best inhibitors for the CEF-RSV system are 5'-deoxy-5'-thio adenosine derivatives having a short 3 to 4 carbon side chain [15]. Fig. 1 and Table 1 show that the isobutyl side chain of SIBA can be replaced by allyl (1b) or methylallyl (2) without loss of activity, whereas the hydroxypropyl group (in 3) gives a less active compound.

SIBA is slowly deaminated in the cells to compound 30 which is much less active [27]. In view of inhibiting deamination and as previous work [28] has shown that a N-6 dimethyl derivative of SIBA is inactive in vitro on a crude tRNA methylase, we have prepared several N-6 mono substituted derivatives (6, 8, 14, 17).

Table 1 shows that the N-6 substitution by a carboxymethyl group as in (14) is a good solution of this problem, better than the substitution by a methyl group, which decreases the activity (6, 8), or by a benzoyl group (as in 1d) which decreases the solubility. Two changes of the structure of SIBA were performed to obtain compound 17 : replacement of the isobutyl group by allyl and the substitution of N-6 of adenine by a carboxy-methyl group. The biological activity of this compound is comparable to that of SIBA, at least on RSV induced cell transformation.

Dialdehyde derivatives of nucleosides, especially of adenosine and of inosine are known to be potent antitumor agents [24, 29, 30]. This prompted us to prepare several analogues of SIBA with modifications in the ribose moiety (21, 25, 26, 27).

Surprisingly we found that the dialdehyde of SIBA 26 is only weakly active whereas its sulfoxide 27 is among the best inhibitors of cell transformation. The dialcohol (21) is inactive and the dioxane 25 is only slightly active.

The S-glucosyl compound 4 was prepared in view of increasing the solubility in water ; it is a good inhibitor but weaker than SIBA. When the S-isobutyl side chain was replaced by N-methyl-isopropyl as in 18, the oncostatic activity was greatly reduced. This was not surprising as the replacement of the sulfur atom of SIBA by a nitrogen atom reduces the activity[15]

To obtain information about the importance of the adenine moiety in the biological effect studied compounds 28 and 29 were synthesized ; both were inactive, 29 seemed even to stimulate cell transformation.

Table 2 shows the ki values for tRNA methylases and protein methylase I of CEF.

Compounds having a ki value for protein methylase I lower than 1000 are good inhibitors of cell transformation, except SAH, which is very rapidly metabolised in normal cells[32]. The ki values found for the tRNA methylases of CEF seem to bear no direct correlation with inhibition of transformation. This might be explained by the fact that we have used a mixture of tRNA methylases whereas a specific inhibitor of one or two of these could be sufficient to inhibit cell transformation*.

A few months ago we received a sample of an antifungal antibiotic from Dr R.S. Gordee (Lilly Research Laboratories, Indianapolis, USA) ; this compound can be considered to be a "carba-analogue" of SAH, as it differs from the latter only in

* The active compounds mentioned in this paper are protected by an ANVAR patent application.

the replacement of the sulfur atom by a $>CH-NH_2$ group. We have found this "sinefungin" to be as active as SIBA in the CEF-RSV test and more active in vitro on tRNA and protein I methylases (M. Vedel, F. Lawrence, M. Robert-Gero, E. Lederer, Biochem. Biophys.Res.Comm., in press). Similar results, showing a very strong in vitro inhibition of mRNA methyltransferases have been published quite recently by Borchardt et al.[31]. Sinefungin is also a potent inhibitor of norepinephrine N-methyltransferase, histamine N-methyltransferase and catechol O-methyltransferase in vitro (R.W. Fuller and R. Nagarajan, personal communication).

Future synthetic efforts will be directed to the synthesis of simplified carba-analogues of SAH.

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