FLUORESCENT ANALOGUES OF ACYCLIC INHIBITORS OF S-ADENOSYL-L-HOMOCYSTEINE HYDROLASE*,**

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Condensation of sodium salt of 2-aminopurine (*I*) with 4-chloromethyl-2,2-dimethyl-1,3-dioxolane (*II*) followed by acid hydrolysis afforded 9-(*RS*)-(2,3-dihydroxypropyl)-2-aminopurine (*V*). Similarly, sodium salt of *lin*-benzoadenine (*IX*) reacted with compound *II* to give 3-(*RS*)-(2,3-dihydroxypropyl)-*lin*-benzoadenine (*Xb*). Analogues of eritadenine (*XVIb*) derived from 2-aminopurine (*VII*) and *lin*-benzoadenine (*XIV*) were obtained by reaction of sodium salt of the corresponding base (*I* or *IX*) with 2,3-O-cyclohexylidene-D-erythronolactone (*VI*) and subsequent acid hydrolysis. By action of chloroacetaldehyde on 9-substituted acyclic analogues of adenosine or AMP (*XVI*) were prepared 9-(2,3-dihydroxypropyl)-1,N^6-ethenoadenine (*XVIIa*), 1,N^6-etheno derivative of eritadenine (*XVIIb*), 3-(1,N^6-ethenoadenin-9-yl)-2-hydroxypropanoic acid (*XVIIc*) and its 2-methylpropyl ester (*XVIId*), as well as 9-(*S*)-(3-hydroxy-2-phosphonylmethoxypropyl)-1,N^6-ethenoadenine (*XVIIe*) and 9-(2-phosphonylmethoxyethyl)-1,N^6-ethenoadenine (*XVIIf*). Fluorescence spectra of all the mentioned compounds exhibit parameters corresponding to the substituted fluorophore; however, no pronounced inhibitory effect on SAH-hydrolase from L-1210 mice leukemia cells has been found for any of them.

In the recent years we have systematically investigated the structure–activity relationship for inhibitors of the important regulatory enzyme S-adenosyl-L-homocysteine hydrolase (SAH-hydrolase) in the region of chiral acyclic nucleoside analogues (for a review see refs[2,3]). Some of these compounds – analogues of adenosine – exhibit a significant antiviral activity[2–5] and a number of other biological effects, caused by inhibition of methylation reactions in proliferating systems (for a review see ref.[2,3,6–9]). Since selected compounds of this group are potential drugs, it is necessary to solve various questions connected with their distribution in the tissues. These inhibitors also offer a potential interesting use in teratology, enabling a study of effects of precisely defined antimetabolic action on embryo malformation as a function of time[8]. In both the mentioned cases the use of fluorescent derivatives,

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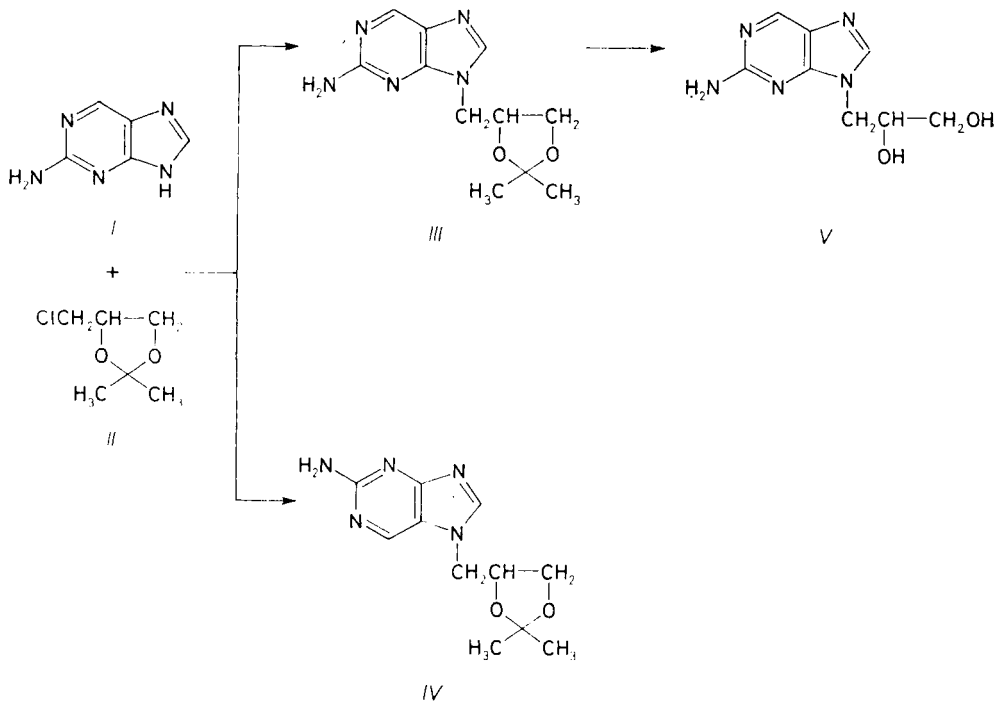
which could replace radionuclide-labelled preparations or allow direct histological studies of distribution in tissues, appeared to be very interesting. Therefore, this study has been aimed at the synthesis of acyclic adenosine analogues with suitable fluorescence properties that would be as effective SAH-hydrolase inhibitors as the corresponding adenine derivatives.

As follows unequivocally from the previous structure–activity studies[10,11], for all types of the mentioned compounds irrespective of the side-chain character any change of the adenine heterocyclic system (substitution in positions 2, 6 and 8) results in a marked drop, or disappearance, of the inhibition effect. This general rule limits considerably the structural variability, since in such type of compounds only a heterocyclic purine base can be the fluorophore. We tried therefore to introduce such minimal changes of the adenine system that could induce sufficiently strong fluorescence without affecting substantially its chemical properties. The choice of the side-chain type was also based on our previous experience: we studied systematically the type of reversible neutral inhibitor[10] (analogues of 9-(2,3-dihydroxypropyl)-adenine, DHPA), the type of acidic irreversible inactivator[11] (analogues of eritadenine or 3-(adenin-9-yl)-2-hydroxypropanoic acid, AHPA), or neutral esters of the latter



SCHEME 1

compound (see refs[5,7]). The reactions employed are based on the principles and synthons used already earlier[2,10,12] or on modification of preformed adenine derivatives.

The first group of compounds studied are derivatives of 2-aminopurine (*I*) which is a sufficiently strong fluorophore (particularly in alkaline media). These compounds are isomeric with biologically active adenine derivatives (vide supra) and have not been studied so far. Since there are only scarce data on the course of alkylation of 2-aminopurine, it was also necessary to establish the structure of the isomer(s) obtained. We studied the preparation of analogues of DHPA and eritadenine, derived from this base. In the first case (Scheme 1), sodium salt of 2-aminopurine was reacted with 4-chloromethyl-2,2-dimethyl-1,3-dioxolane (*II*) in dimethylformamide at elevated temperature (analogously to alkylation of adenine[13]) under formation of two compounds. The reaction gave predominantly the N^9-isomer *III* along with minor quantities of the N^7-isomer *IV*. Acid hydrolysis of compound *III* afforded 9-(*RS*)-(2,3-dihydroxypropyl)-2-aminopurine (an analogue of the adenine antiviral DHPA[2,3]).

The structure of products *III* and *IV* has been unequivocally determined from the APT (attached proton test) spectra, undecoupled ^{13}C NMR spectra and the known[14,15] differences in the C-4 and C-5 chemical shifts for the N^9- and N^7-substituted isomers. For both compounds the carbon signals of the base occur in the low-field region (δ 120–160 ppm). The APT spectra identified the C-6 and C-8 methine carbon signals which were then assigned on the basis of their ^1J(C, H) coupling constants in the proton-coupled ^{13}C NMR spectra. For the N^9-derivative *III* the C-6 signal appears as a doublet at δ 149·68 with ^1J(C-6, H-6) = 181·2 Hz and the C-8 signal as a doublet at δ 143·38, ^1J(C-8, H-8) = 211·6 Hz. Of the quarternary carbon signals, the C-2 doublet occurs at the lowest field (δ 159·88; ^3J(C-2, H-6) = 11·6 Hz) whereas the C-4 and C-5 carbon atoms give rise to unresolved multiplets at δ 153·30 (C-4) and δ 127·74 (C-5). Both signals are split due to long-range coupling with H-6 and H-8. The carbon atoms in the N^7-isomer *IV* were assigned analogously; in accord with the literature, its C-4 signal is shifted 9·46 ppm downfield and the C-5 signal 7·51 ppm upfield as compared with the N^9-isomer *III*. All the remaining carbon signals of the base are also shifted: the chemical shifts $\Delta\delta = \delta(N^7\text{-derivative}) - \delta(N^9\text{-derivative})$ are given in Table I. Analogous results have been obtained with the isomeric pair *VII* and *VIII*, where the H-8 and H-1′ proton signals in the spectra of the N^7-derivative are shifted downfield relative to those of the N^9-isomer.

The alkylation course of 2-aminopurine (*I*) thus differs from the analogous reaction of adenine with compound *II* (see ref.[13]) where the N^3-isomer of DHPA instead of the N^7-isomer is the minor side product. Compounds *III* (or *V*) and *IV* exhibit characteristic UV spectra (see Experimental and Table II). This fact was utilized in structural assignment of the second group of 2-aminopurine derivatives prepared

TABLE I

^{13}C NMR chemical shifts and coupling constants (in parentheses)

Compound	C-2 (^3J(C-2, H-6))	C-4	C-5	C-6 (^1J(C-6, H-6))	C-8 (^1J(C-8, H-8))	C-1′ (^1J(C-1′, H-1′))	C-2′ (^1J(C-2′, H-2′))	C-3′ (^1J(C-3′, H-3′))
III[a]	159·88 (11·6)	153·30	127·74	149·68 (191·2)	143·38 (211·6)	44·38 (141·1)	73·78 (152·9)	66·36 (147·7)
IV[a]	160·36 (11·7)	162·76	120·23	141·81 (182·5)	147·75 (209·3)	51·27 (140·8)	74·23 (152·2)	66·31 (148·9)
V[b]	159·87	153·18	136·91	149·09	145·44	45·90	63·02	69·95
VII[b]	159·87	153·23	126·92	148·94	145·56	44·96	71·09	74·06
VIII[b]	160·13	161·43	120·25	143·29	149·78	47·93	71·70	74·11
$\Delta\delta$(*IV*−*III*)	0·48	9·46	−7·51	−7·87	4·37	6·43	−0·55	−0·05
$\Delta\delta$(*VIII*−*VII*)	0·26	8·20	−6·67	−5·66	4·22	2·97	0·61	0·05

[a] Solvent $CDCl_3$; [b] solvent D_2O.

within the framework of this study: analogues of the natural compound eritadenine, *erythro*-(2*R*,3*R*)-4-(adenin-9-yl)-2,3-dihydroxybutanoic acid, a potent irreversible SAH-hydrolase inactivator[11]. By analogy with the synthesis of eritadenine[17,18], its 2-aminopurine analogue was prepared by condensing sodium salt of base *I* with 2,3-O-cyclohexylidene-D-erythronolactone (*VI*) in dimethylformamide (Scheme 2). Acidic work-up of the crude product gave both (9- and 7-) isomers *VII* and *VIII* which were assigned structure using the UV and NMR spectra (vide supra).

An interesting alternative for fluorescent analogues of purine derivatives has been suggested by Leonard and coworkers[19]: transformation of purine bases into their so-called *lin*-benzo derivatives of the type *IX* changes only slightly the mutual distances between groups in the molecule and retains the planarity of the heteroaromatic ring. The hydrophobicity of the system is higher and the analogues exhibit a strong fluorescence. A number of enzymes, interacting with purine nucleosides and nucleotides, have been found to interact also with their *lin*-benzo analogues[19]. The acyclic adenosine analogues, containing the *lin*-benzoadenine instead of adenine system, were synthesized similarly as the 2-aminopurine derivatives: sodium salt of *lin*-benzoadenine[20] (*IX*) was condensed with the synthon *II* to give two isomeric 1,3-dioxolane derivatives *Xa* and *XIa*. Proton NMR spectra have proven the struc-

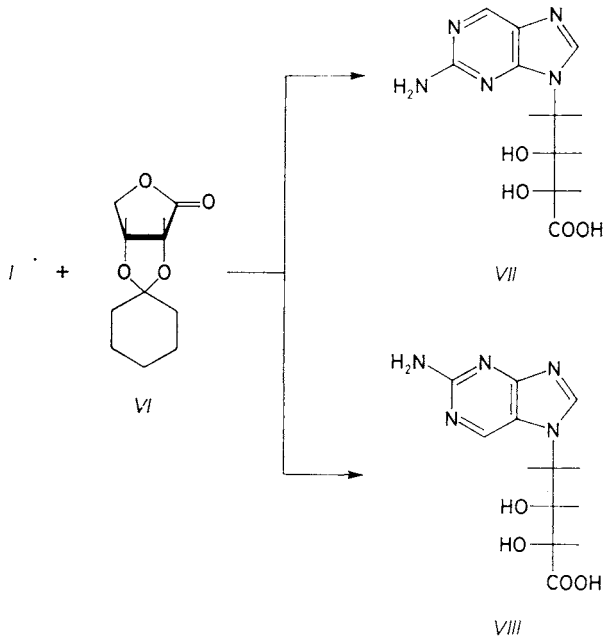
TABLE II

Properties of fluorescent acyclic nucleoside analogues

Compound	UV-spectra[a]		Fluorescence spectra[a]		v_i/v_o[b]
	λ_{max}, nm	$\varepsilon_{max} \cdot 10^{-3}$	λ_{max}, nm	quantum yield (+)	
V	305; 240	6·8; 5·5	298	0·50	1·00
VII	305; 240	6·9; 4·9	298	0·52	0·96
VIII	318; 256	5·7; 3·7	—	—	—
Xb	348; 332; 317	7·8; 10·4; 9·1	330	0·27	1·02
XIV	348; 332; 318	7·7; 10·3; 8·4	330	0·18	0·94
XVIIa	294; 276; 266	3·4; 6·1; 5·9	298	0·56	0·83
XVIIb	294; 276; 266	3·3; 6·1; 5·9	298	0·45	0·57
XVIIc	294; 276; 266	3·3; 6·1; 6·0	298	0·54	0·94
XVIId	298; 276; 266	3·3; 6·1; 5·9	298	0·57	0·69
XVIIe	298; 276; 266	2·4; 5·4; 5·2	298	0·27	—
XVIIf	294; 276; 266	3·2; 5·6; 5·4	298	0·30	—

[a] In 0·03M sodium phosphate buffer pH 7·0; [b] L-1210 SAHase inhibition[16]; initial rate of SAH hydrolysis in the presence (v_i) or absence (v_o) of inhibitor; [SAH] = 8 . 10^{-6} mol l^{-1}; [I]/[S] = = 0·125. (*S*)-DHPA: v_i/v_o = 0·34.

ture of the products *Xa* and *XIa* as well as their respective N,N-dibenzoyl derivatives *XII* and *XIII*, prepared by benzoylation.

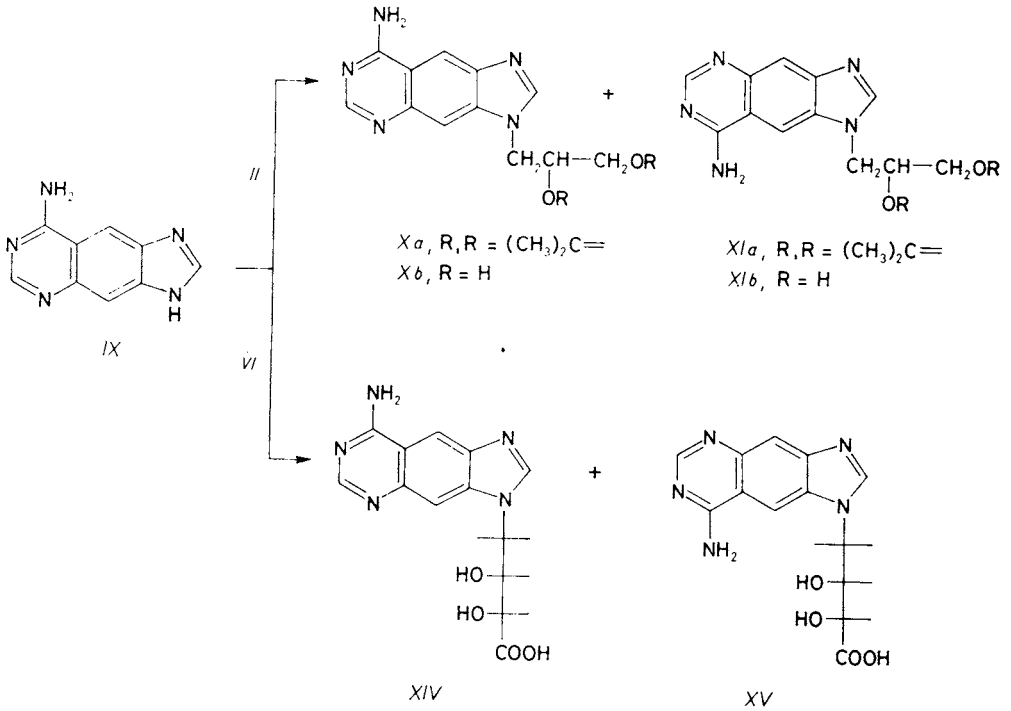


SCHEME 2

Acid deblocking afforded the isomeric 2,3-dihydroxypropyl derivatives *Xb* and *XIb* (Scheme 3), homogeneous according to all criteria of purity. Condensation of sodium salt of *IX* with protected D-erythronolactone *VI*, followed by acidic deprotection, also afforded a mixture of the 3- and 1-isomer *XIV* and *XV*. This mixture could not be completely separated even by preparative HPLC. Only after conversion of *XIV* and *XV* into 2,3-isopropylidene derivatives of their esters, repeated chromatography on silica gel and subsequent deprotection, the desired pure isomer *XIV* was obtained in low yield. Its structure was established on the basis of the UV spectrum which agreed with that of compound *Xb* (whose structure was determined by NMR spectroscopy) as well as with the published[21] data for *lin*-benzoadenosine.

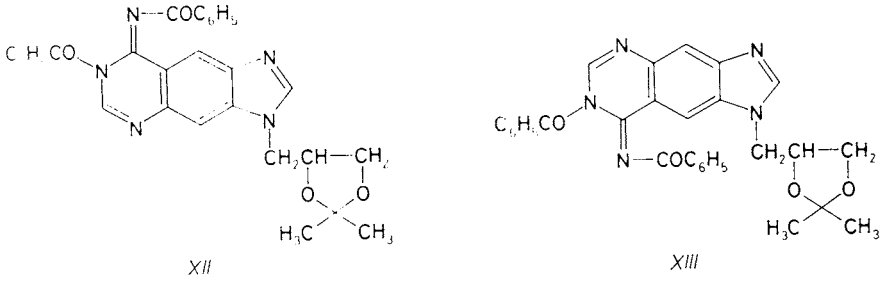
The third group of fluorophore-containing acyclic analogues of nucleosides are the so-called 1,N^6-ethenoadenine derivatives *XVII* which, however, are devoid of the 6-amino group, characteristic of the adenine system. These compounds were readily obtained by reaction of the corresponding adenine derivatives *XVI* with chloroacetaldehyde in weakly acidic aqueous medium[22]. We prepared four etheno

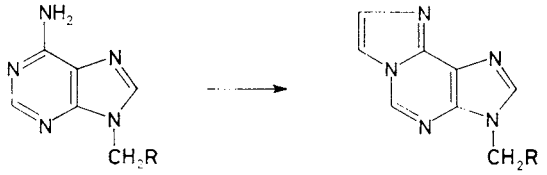
derivatives (*XVII*) of the most often studied typical SAH-hydrolyse inhibitors: DHPA (*XVIIa*), AHPA (*XVIIc*), eritadenine (*XVIIb*), and 2-methylpropyl ester of AHPA (*XVIId*). All the reactions were practically quantitative. Products *XVII* were



SCHEME 3

freed from salts and traces of the starting compounds by chromatography on a column of octadecylsilica gel in an aqueous medium (Scheme 4).





XVI *XVII*

In formulae *XVI* and *XVII*: *a*, R = —CH(OH)CH_2OH *b*, R = (2*R*,3*R*)—CH(OH)CH(OH)COOH
c, R = —CH(OH)COOH *d*, R = —CH(OH)$COOCH_2CH(CH_3)_2$
e, R = (*S*)—$CH(CH_2OH)OCH_2P(O)(OH)_2$ *f*, R = —$CH_2OCH_2P(O)(OH)_2$

SCHEME 4

The series of 1,N^6-etheno derivatives *XVII* was extended by fluorescent analogues of two other biologically important adenine derivatives: 9-(*S*)-(3-hydroxy-2-phosphonylmethoxypropyl)adenine (HPMPA) (*XVIIe*) and 9-(2-phosphonylmethoxyethyl)adenine (PMEA) (*XVIIf*). Both HPMPA and PMEA are novel antivirals, effective against DNA viruses[23]. Their sodium salts[24,25] reacted easily with chloroacetaldehyde in aqueous solutions and after isolation on octadecylsilica gel afforded in high yields the 1,N^6-etheno derivatives *XVIIe* and *XVIIf*, homogeneous according to HPLC.

Fluorescence Studies

The spectroscopic measurements (Fig. 1) confirmed the expected fluorescence values for all the prepared compounds. The quantum yields (Table II) correspond to the

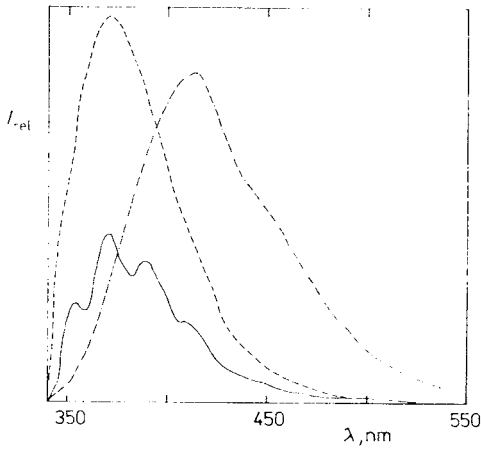


FIG. 1

Corrected fluorescent emission spectra of three types of fluorophores (2,3-dihydroxypropyl derivatives) in aqueous buffer solution (0·03M phosphate) at concentrations (3·2 to 9·6) . 10^{-6} mol l^{-1}; (— — —) *V*, (———) *Xb*, and (– · – · –) *XVIIa*

data given for other derivatives of these fluorophores. The N^9-isomers of 2-aminopurine derivatives (ethyl, β-D-ribofuranosyl, 2-deoxy-β-D-ribofuranosyl) have absorption maxima at 303–304 nm and the substitution at the N^9-position of the base has no marked effect on the quantum yield (Q = 0·68) of this fluorophore[26]. The values found for compounds *V* and *VII* (Q = 0·50–0·52) are in accord with this finding. For 1,N^6-etheno derivatives of adenine nucleosides and nucleotides the reported[22] quantum yield Q is 0·56 and the absorption maximum is at 275 nm. Our values for compounds *XVIIa–d* range from 0·45 to 0·57. Lower quantum yields were found only for the phosphonylmethyl derivatives *XVIIe* and *XVIIf*; also the *lin*-benzoadenine derivatives *Xb* and *XIV* had lower values than reported[21] for *lin*-benzoadenosine (Q = 0·44). However, molar extinction coefficients of compounds *XVIIe*, *XVIIf*, *Xb*, and *XIV*, as well as the general characteristics of their UV spectra, correspond to the data for 9-substituted derivatives of 1,N^6-ethenoadenine or 3-substituted derivatives of *lin*-benzoadenine.

Inhibition of SAH-*hydrolase in vitro*

The inhibition properties of the fluorescent derivatives *V*, *VII*, *Xb*, *XIV*, and *XVIIa–d*, prepared in this work, were studied in a standard in vitro test using hydrolysis of S-adenosyl-L-homocysteine[16] with a purified enzyme from L-1210 mice leukemia cells[27]. As control material we used 9-(*S*)-(2,3-dihydroxypropyl)adenine (*XVIa*) (see ref.[16]). The results, summarized in Table II, show that the inhibitory effect of compounds derived from 2-aminopurine (*V*, *VII*), *lin*-benzoadenine (*Xb*, *XIV*) and 1,N^6-ethenoadenine (*XVIIa–d*) is marginal compared with the corresponding adenine derivatives *XVI* (see refs[10,11]). Some activity was found only with the 1,N^6-etheno derivative *XVIIb*; nevertheless, even in this case the activity was by an order of magnitude lower than that reported[11] for eritadenine (*XVIb*) and even for (*S*)-*DHPA* (*XVIb*)[10]. Although these results have not fulfilled our expectations, they again underline the importance of an intact adenine system as a necessary structural condition for the interaction of an analogue in the binding site of SAH-hydrolase[10,11].

The fluorescent derivatives *V*, *VII*, *Xb*, *XIV*, and *XVIIa–f* were also tested for antiviral activity in vitro. In concentrations up to 200 mg/l (i.e. about 1 mmol l^{-1}) they had no inhibitory effect on vesicular stomatitis virus, herpes simplex type 1 and 2 viruses and vaccinia virus.

EXPERIMENTAL

Unless stated otherwise, the solutions were evaporated at 40°C/2 kPa and the compounds were dried over phosphorus pentoxide at 13 Pa. Melting points were determined on a Kofler block and are uncorrected. Thin-layer chromatography (TLC) on silica gel was performed on Silufol

UV 254 plates (Kavalier, Votice, Czechoslovakia) in the systems S1 chloroform–methanol (95 : 5), S2 chloroform–methanol (9 : 1), S3 chloroform–methanol (8 : 2), S4 chloroform–methanol (7 : 3), S5 acetone–water–ethanol–ethyl acetate (4 : 1 : 1 : 1). Preparative TLC was done on loose layers (50 × 16 × 0·3 mm) of silica gel with a fluorescent indicator (Silpearl, Kavalier, Votice, Czechoslovakia). Paper chromatography was carried out on a Whatman No. 1 paper in the system S6 2-propanol–conc. aqueous ammonia–water (7 : 1 : 2). HPLC analyses were performed on a 4 × 200 mm column of Silasorb C18 (5 μ), flow rate 0·4 ml min^{-1}, in 0·05M triethylammonium hydrogen carbonate, pH 7, containing: 4% methanol (S7), 5% methanol (S8), 20% methanol (S9), 2% acetonitrile (S10), 6·6% acetonitrile (S11), 15% acetonitrile (S12), 0·1% trifluoroacetic acid (S13), and 10% methanol + 0·1% trifluoroacetic acid (S14). Detection at 254 nm. Preparative chromatography on silica gel was performed with silica gel according to Pitra (30—40 μ, Service Laboratories of this Institute), chromatography on octadecylsilica gel on a 20 × 3 cm column of sorbent prepared by modification of Silpearl (30 μ). Paper electrophoresis was carried out on a Whatman No. 3MM paper at 20 V/cm (1 h) in 0·05 mol l^{-1} triethylammonium hydrogen carbonate, pH 7·5 (E1).

Ultraviolet absorption spectra were measured on a PU 8 800 UV/VIS spectrophotometer (Pye Unicam, Cambridge, Great Britain) in a sodium phosphate buffer, pH 7. The fluorescence spectra were taken on a Fluorolog 211 instrument with a 450 W lamp (SPEX Industries, U.S.A.) in a sodium phosphate buffer, pH 7. The quantum yield Q was calculated according to the formula[27] $Q = A'(F/F')\,(A'/A)$, where $Q = 0{\cdot}70$ for quinine sulfate, F/F' is the ratio of integrals of corrected emission spectra and A'/A is the ratio of absorbancies of the fluorophores at the excitation wavelengths (absorbancy of quinine sulfate at 366 nm $A' = 0{\cdot}020$). ^1H and ^{13}C NMR spectra were taken on a Varian XL-200 instrument (200 MHz) in deuteriochloroform, hexadeuteriodimethyl sulfoxide or deuterium oxide with tetramethylsilane as internal standard; chemical shifts are given in ppm, coupling constants in Hz.

9-(*RS*)-(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl-2-aminopurine (*III*) and
7-(*RS*)-(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl-2-aminopurine (*IV*)

Sodium hydride (480 mg; 20 mmol) was added to a suspension of 2-aminopurine (*I*, dried over phosphorus pentoxide at 13 Pa; 2·7 g; 20 mmol) in dimethylformamide (60 ml). The mixture was stirred at 80°C for 1 h (calcium chloride protective tube). After addition of 2,2-dimethyl-4-chloromethyl-1,3-dioxolane (*II*; 5 ml), the stirred mixture was heated to 100°C for 14 h (calcium chloride tube). The solvent was evaporated at 50°C/2 kPa, the residue was codistilled with toluene (2 × 50 ml) under the same conditions and extracted with boiling chloroform (500 ml total). The extract was taken down in vacuo and the residue was chromatographed on a column (400 ml) of silica gel, yielding 2·2 g (44%) of the 9-isomer *III*, m.p. 183—184°C (ethanol–light petroleum). For $C_{11}H_{15}N_5O_2$ (249·3) calculated: 53·00% C, 6·06% H, 28·10% N; found: 52·62% C, 5·99% H, 27·72% N. $R_F = 0{\cdot}62$ (S3). ^1H NMR ($CDCl_3$): 8·66 s and 7·85 s (2 arom. H); 4·43 qd (CH—O); 4·26 dd and 4·12 dd (CH_2N, $^2J = 14{\cdot}4$, $^3J = 6{\cdot}0$ and 3·8); 4·06 dd and 3·68 dd (CH_2O, $^2J = 8{\cdot}8$, $^3J = 6{\cdot}5$ and 6·0); 1·34 and 1·30 (2 × CH_3). UV (pH 7) λ_{max} (ε_{max}): 306 (5 780), 244 (4 000).

Further fractions gave 0·75 g (15%) of the 7-isomer *IV*, m.p. 178—178°C (ethanol–light petroleum). For $C_{11}H_{15}N_5O_2$ (249·3) calculated: 53·00% C, 6·06% H, 28·10% N; found: 53·40% C, 5·79% H, 27·70% N. $R_F = 0{\cdot}44$ (S3). ^1H NMR ($CDCl_3$): 8·60 s and 8·02 s (2 arom. H); 4·42 m (CH—O); 4·25 m (CH_2N); 4·08 m and 3·68 dd (CH_2O; $^2J = 8{\cdot}6$ and $^3J = 6{\cdot}0$); 1·38 s and 1·34 s (2 × CH_3); $(CD_3)_2SO$: 8·67 s and 8·21 s (2 arom. H); 6·15 br s (NH_2). UV (pH 7) λ_{max} (ε_{max}): 318 (5 690), 256 (3 750).

9-(*RS*)-(2,3-Dihydroxypropyl)-2-aminopurine (*V*)

A solution of *III* (850 mg; 3·4 mmol) in 0·25 mol l^{-1} sulfuric acid (25 ml) was set aside at room temperature overnight, diluted with water (100 ml) and neutralized (to pH 7) with a saturated aqueous solution of barium hydroxide. The suspension was heated to the boil, filtered through Celite and the material on the filter was washed with boiling water (500 ml). The filtrate was evaporated in vacuo, the residue was codistilled with ethanol (100 ml) and crystallized from 80% ethanol (with ether added to turbidity) to afford 0·70 g (90·3%) of compound *V* as the monohydrate, m.p. 156–157°C. For $C_8H_{13}N_5O_3$ (227·3) calculated: 42·28% C, 5·76% H, 30·83% N; found: 42·57% C, 5·39% H, 30·53% N. R_F = 0·57 (S6). HPLC (S8): k = 5·40.

erythro-(2*R*,3*R*)-4-(2-Aminopurin-9-yl)-2,3-dihydroxybutanoic Acid (*VII*) and
erythro-(2*R*,3*R*)-4-(2-Aminopurin-7-yl)-2,3-dihydroxybutanoic Acid (*VIII*)

A mixture of 2-aminopurine (*I*; 1·35 g; 10 mmol), dimethylformamide (30 ml) and 60% sodium hydride dispersion (0·40 g; 10 mmol) was stirred at 80°C for 1 h (reflux condenser with calcium chloride protective tube). 2,3-O-Cyclohexylidene-D-erythronolactone (*VI*; 3·75 g; 18 mmol) was added and the mixture was heated to 100°C for 24 h until the starting compound disappeared (monitored by electrophoresis in E1). After evaporation of the solvent in vacuo, the residue was codistilled with toluene. The residue was refluxed with 85% formic acid (50 ml) for 2 h, the acid was evaporated in vacuo and the crude mixture was deionized on Dowex 50 (H^+). Elution with water removed salts and the unreacted lactone, further elution with 20% aqueous ammonia afforded the crude condensation products *VII* and *VIII*. After evaporation, this mixture was separated on a column (150 ml) of Sephadex A-25. Elution with 0·02M triethylammonium hydrogen carbonate removed the unreacted base and gradient elution (0·02–0·2M) with the same buffer (à 1 l) afforded a mixture of isomers which were obtained pure by preparative HPLC in 50 mM triethylammonium hydrogen carbonate. The pure isomers were converted into the sodium salts on Dowex 50 (Na^+ form); codistillation with ethanol and crystallization from ethanol–ether gave 0·30 g (9·6%) of the 9-isomer *VII*. For $C_9H_{10}N_5O_4Na.2\,H_2O$ (311·2) calculated: 34·73% C, 4·53% H, 22·49% N; found: 35·04% C, 4·51% H, 22·03% N. HPLC (S8): k = 3·3. ^{1}H NMR (D_2O): 8·63 s and 8·16 s (2 arom. H); 4·43–4·30 m, 3 H (CH_2N and CH—O); 4·23 d (CH—O, ^3J = 3·4). $[\alpha]_D^{20}$ +19·8 (*c* 0·5, 0·1M HCl).

The 7-isomer *VIII* was obtained in 3·2% yield (0·10 g). For $C_9H_{10}N_5O_4Na.2\,H_2O$ (311·2) calculated: 34·73% C, 4·53% H, 22·49% N; found: 34·53% C, 4·04% H, 22·30% N. HPLC (S8): k = 1·2. ^{1}H NMR (D_2O): 8·77 s and 8·37 s (2 arom. H); 4·47–4·31 m, 3 H (CH_2N and CH—O); 4·23 d (CH—O, ^3J = 3·5). $[\alpha]_D^{20}$ +13·3° (*c* 0·5, 0·1M HCl).

3-(*RS*)-(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl-*lin*-benzoadenine (*Xa*) and
1-(*RS*)-(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl-*lin*-benzoadenine (*XIa*)

Sodium hydride dispersion (60% in mineral oil; 0·28 g; 7 mmol) was added to a suspension of *lin*-benzoadenine (*IX*; dried by codistillation with pyridine; 1·3 g; 7 mmol) in dimethylformamide (35 ml). After sonication for 30 min and stirring at 125°C for 30 min (calcium chloride protective tube), 2,2-dimethyl-4-chloromethyl-1,3-dioxolane (*II*; 4 ml) was added. The mixture was heated to 125°C (calcium chloride tube) until the starting compound disappeared (TLC in S4; 16 h), and filtered while hot through Celite which was then washed several times with hot dimethylformamide. The filtrate was taken down in vacuo, the residue was codistilled twice with toluene, adsorbed on silica gel (20 g) and chromatographed on a column of silica gel (300 ml). Yield 0·88 g (42%) of the 3-isomer *Xa*, m.p. >250°C (ethanol–ethyl acetate–light petroleum). For

$C_{15}H_{17}N_5O_2$ (299·3) calculated: 60·19% C, 5·73% H, 23·39% N; found: 59·72% C, 5·49% H, 23·01% N. R_F = 0·72 (S4). ^{1}H NMR ($(CD_3)_2SO$): 8·68 s, 8·45 s, 8·43 s, and 7·92 s (4 arom. H); 7·99 bs (NH_2); 4·62–4·34 m (CH_2N, CH—O); 4·12 dd and 3·73 dd (CH_2O, ^2J = 8·7, ^3J = 6·3 and 5·6); 1·28 s and 1·25 s (2 × CH_3).

Further elution gave 0·27 g (13%) of the 1-isomer *XIa*; m.p. <250°C (ethanol–ethyl acetate–light petroleum). For $C_{15}H_{17}N_5O_2$ (299·3) calculated: 60·19% C, 5·73% H, 23·39% N; found: 59·60% C, 5·55% H, 23·11% N. R_F = 0·55 (S4). ^{1}H NMR ($(CD_3)_2SO$): 8·91 s, 8·63 s, 8·57 s, and 8·05 s (4 arom. H); 8·77 bs (NH_2); 4·64 m (CH—O, ^3J = 6·8, 6·4, 5·4, and 4·0); 4·54 dd and 4·43 dd (CH_2N, ^2J = 14·2, ^3J = 6·8 and 4·0); 4·12 dd and 3·80 dd (CH_2O, ^2J = 8·7, ^3J = 6·4 and 5·4); 1·29 s and 1·32 s (2 × CH_3).

3-(*RS*)-(2,3-Dihydroxypropyl)-*lin*-benzoadenine (*Xb*)

A mixture of *Xa* (0·88 g; 2·9 mmol) and 80% acetic acid (130 ml) was refluxed for 1 h. After evaporation of acetic acid in vacuo and codistillation with water, the residue was crystallized from 80% ethanol (ether added to turbidity) to afford 0·3 g (37·5%) of *Xb* as the monohydrate m.p. <250°C. For $C_{12}H_{13}N_5O_2.H_2O$ (277·2) calculated: 51·99% C, 5·45% H, 25·25% N; found: 52·00% C, 5·01% H, 25·03% N. R_F = 0·56 (S6), HPLC (S9): k = 3·0. ^{1}H NMR ($(CD_3)_2SO$): 8·64 s, 8·40 s, 8·35 s, and 7·87 s (4 arom. H); 7·71 bs (NH_2); 4·47 dd and 4·24 dd (N—CH_2, ^2J = 14·4, ^3J = 3·4 and 7·5); 3·91 m (CH—OH, w = 22 Hz); 3·49 dd and 3·37 dd (CH_2—OH, ^2J = 10·9, ^3J = 5·0 and 6·5). ^{13}C NMR ($(CD_3)_2SO$): 162·7, 145·3, 142·7, 139·2 (4 arom. —C=); 153·9, 149·6, 113·3, 106·2 (4 arom. —CH=); 70·1 (CH—OH); 63·5 (CH_2OH); 48·0 (CH_2N).

Dibenzoyl Derivative *XII*

Benzoyl chloride (0·4 ml; 3·4 mmol) was added to an ice-cooled stirred mixture of *Xa* (0·215 g; 10·7 mmol) and dry pyridine (5 ml). The obtained clear solution was stirred under cooling for 1 h and at room temperature for 18 h (calcium chloride tube). Methanol (5 ml) was added, followed by ethyl acetate (50 ml). The mixture was washed with water (3 × 20 ml), ethyl acetate was evaporated in vacuo and the residue was codistilled three times with toluene and chromatographed on a loose layer of silica gel in chloroform containing 5% of methanol. Yield 0·20 g (55·5%) of the perbenzoyl derivative *XII*, m.p. 143–145°C (ethyl acetate–ether–light petroleum). For $C_{29}H_{25}N_5O_4$ (507·5) calculated: 68·62% C, 4·97% H, 13·79% N; found: 68·37% C, 4·78% H, 13·38% N. R_F = 0·41 (S2). ^{1}H NMR ($CDCl_3$): 8·95 s, 8·55 s, 8·27 s, and 8·05 s (4 arom. H); 7·90 m, 4 H and 7·31–7·53 m, 6 H (2 × COC_6H_5); 4·51 m (CH—O, ^3J = 6·8, 6·4, 6·0, and 3·2); 4·39 dd and 4·25 dd (CH_2N, ^2J = 14·6, ^3J = 3·2 and 6·8); 4·14 dd and 3·70 dd (CH_2O, ^2J = 8·7, ^3J = 6·4 and 6·0); 1·40 s and 1·32 s (2 × CH_3).

Dibenzoyl Derivative *XIII*

Benzoyl chloride (0·4 ml) was added to a stirred and ice-cooled mixture of *XIa* (0·3 g; 1 mmol) and dry pyridine (5 ml). The clear solution was stirred under cooling for 1 h and then at room temperature for 18 h (calcium chloride tube). After addition of methanol (5 ml) and ethyl acetate (50 ml) the mixture was washed with water (3 × 20 ml). Ethyl acetate was evaporated in vacuo and the yellow residue was codistilled with toluene and chromatographed on a loose layer of silica gel in chloroform with 8% of methanol. Yield 0·15 g (30%) of *XIII*, m.p. 222–225°C. For $C_{29}H_{25}N_5O_4$ (507·5) calculated: 68·62% C, 4·97% H, 13·97% N; found: 68·19% C, 4·67% H, 13·86% N. R_F = 0·34 (S2). ^{1}H NMR ($CDCl_3$): 9·02 s, 8·57 s, 8·44 s, and 8·03 s (4 arom. H);

7·87 m, 4 H and 7·31—7·54 m, 6 H (2 × COC_6H_5); 4·41 (CH—O, ^3J = 6·9, 6·5, 6·2, and 3·2); 4·37 dd and 4·23 dd (CH_2N, ^2J = 14·8, ^3J = 3·2 and 6·9); 4·05 and 3·61 dd (CH_2O, ^2J = 8·6, ^3J = 6·2 and 6·5); 1·27 s and 1·25 s (2 × CH_3).

erythro-(2*R*,3*R*)-4-(*lin*-Benzoadenin-3-yl)-2,3-dihydroxybutanoic Acid (*XIV*)

Sodium hydride dispersion (60%; 0·28 g; 7 mmol) was added to a suspension of *lin*-benzoadenine[17,18] (*IX*; 1·3 g; 7 mmol; dried by codistillation with pyridine) in dimethylformamide (36 ml). The mixture was sonicated for 30 min and then stirred at 125°C for 30 min (calcium chloride tube). After addition of 2,3-O-cyclohexylidene-D-erythronolactone (*VI*; 3·5 g; 17·5 mmol) the mixture was heated to 125°C for 16 h (calcium chloride tube) until the starting compound disappeared (monitored by electrophoresis in E1). The hot reaction mixture was filtered through Celite which was then washed several times with hot dimethylformamide. The filtrate was taken down in vacuo, the residue was twice codistilled with toluene and refluxed with 85% formic acid (50 ml) for 2 h. After evaporation of the formic acid and codistillation with water, the crude mixture was deionized on Dowex 50 (H^+ form). Elution with 20% aqueous methanol removed the salts and the unreacted lactone. Further elution with methanol–ammonia–water (2 : 1 : 7) afforded a mixture of the unreacted *lin*-benzoadenine and condensation products *XIV* and *XV*. After evaporation in vacuo, this mixture was separated on column of Sephadex A-25 (150 ml). The unreacted base was separated by elution with 0·02M triethylammonium hydrogen carbonate in 20% methanol (pH 7·5). Gradient elution with the same buffer (0·02—0·2M) in 50% ethanol gave a mixture of *XIV* and *XV*. These compounds were separated by preparative HPLC (water–methanol: 0—15 min 10% methanol, 15—35 min: gradient 1% methanol/min). Conversion into the sodium salts on Dowex 50 (Na^+ form) gave 0·49 g (19·4%) (ethanol–ether) of pure mixture of the N-3 and N-1 isomers *XIV* and *XV* in the ratio 5 : 4 (^{1}H NMR spectrum). For $C_{13}H_{12}N_5O_4Na.2\,H_2O$ (361·2) calculated: 43·22% C, 3·91% H, 19·38% N; found: 43·64% C, 4·02% H, 19·54% N. HPLC (S14): k_1 = 4·2, k_2 = 3·3.

Attempted separation of isomers XIV *and* XV. The ethyl esters (prepared as described previously[5]) were converted into the isopropylidene derivatives which were first separated on a column of silica gel and then several times on a preparative loose layer of silica gel. Acid hydrolysis afforded a pure (^{1}H NMR) isomer identified by UV spectrum[20] as the N-3 isomer *XIV* (Table II).

1,N^6-Etheno Derivatives *XVIIa–f*

A mixture of the adenine derivative *XVI* (2 mmol) and 1M aqueous solution of chloroacetaldehyde[22] (30 ml) (pH 4·5) was kept at 40°C until the starting compound disappeared (TLC in S5). The mixture was taken down and the residue was dissolved in water (5 ml) and applied onto a column of octadecylsilica gel. The product was eluted with water (in the case of *XVIIa* with 10% aqueous methanol). The elution was monitored continuously using a conductometric detector and a Uvicord (LKB, Sweden) instrument. In the cases of compounds *XVIIa,c,d,e,f* the procedure gave HPLC-homogeneous products. Compound *XVIIb* was purified by preparative HPLC (0·1% trifluoroacetic acid). The products *XVIIb,c,e,f* were converted into the sodium salts on Dowex 50 (Na^+ form). Analytically pure samples were obtained by crystallization from ethanol — ether (*XVIIa*), methanol — ether and light petroleum (*XVIIb,c,e,f*), and water (*VIId*). The reaction times, yields, melting points, elemental analyses and HPLC systems are given in Table III.

XVIIa: ^{1}H NMR ($(CD_3)_2SO$): 9·25 s, 8·19 s, 8·05 d, and 7·52 d (4 arom. H, ^3J = 1·5); 5·10 d, (CH—OH, ^3J = 5·4); 4·83 t (CH_2—OH, ^3J = 5·6); 4·46 dd and 4·15 dd (CH_2N, ^2J = 13·8, ^3J = 3·4 and 8·3); 3·89 m (CH—OH, ^3J = 8·3, 5·8, 5·8, 5·4, and 3·4); 3·42 m (CH—OH).

TABLE III

Properties of 1,N^6-etheno derivatives *XVIIa–f*

Compound react. time, h	Yield, % (m.p., °C)	Formula (mol. wt.)	Calculated/found				HPLC system *k*	$[\alpha]_D^{20a}$
			% C	% H	% N	% P		
XVIIa	89·6	$C_{10}H_{11}N_5O_2.\frac{1}{2}H_2O$	49·59	4·99	28·90	—	S7	−39·8
24	(240–244)	(242·2)	49·78	4·69	28·49	—	7·63	
XVIIb	28·5	$C_{11}H_{10}N_5O_4Na.3\,H_2O$	37·40	4·57	19·82	—	S13	+13·8
96	(—)	(353·2)	37·47	4·42	19·90	—	8·24	
XVIIc	42·8	$C_{10}H_8N_5O_3Na.H_2O$	41·82	3·51	24·37	—	S13	—
24	(—)	(287·2)	41·92	3·09	24·08	—	6·65	
XVIId	76·4	$C_{14}H_{17}N_5O_3.H_2O$	52·33	5·96	21·78	—	S12	—
18	(60–70)	(321·3)	52·22	5·85	21·89	—	6·11	
XVIIe	48·6	$C_{11}H_{12}N_5O_5Na_2P$	—	—	18·86	8·34	S11	−23·8
48	(—)	(371·2)	—	—	19·25	8·46	1·91	
XVIIf	78·1	$C_{10}H_{10}N_5O_4Na_2P.H_2O$	—	—	19·48	8·63	S10	—
24	(—)	(359·2)	—	—	19·58	9·34	2·17	

[a] $c = 0{\cdot}5$, 0·1M-HCl.

XVIIb: ^{1}H NMR (D_2O): 8·81 s, 8·08 s, 7·78 bs, and 7·42 bs (4 arom. H); 4·35 bs, 3 H (CH_2N and CH—O); 4·20 bs (CH—O).

XVIIc: ^{1}H NMR (D_2O): 8·82 s, 8·07 s, 7·71 bs, and 7·41 bs (4 arom. H); 4·64—4·32 m, 3 H (CH_2N and CH—O).

XVIId: ^{1}H NMR ($(CD_3)_2SO$): 8·85 s, 8·10 s, 7·76 d, and 7·52 d (4 arom. H, ^3J = 1·5); 4·78 dd (CH—OH, ^3J = 8·1 and 3·4); 4·76 dd and 4·52 dd (CH_2N, ^2J = 14·5, ^3J = 3·4 and 8·1); 3·98 dd and 3·92 dd (CO—CH_2, ^2J = 10·6, ^3J = 6·8 and 6·7); 1·98 m (—CH=, ^3J = 6·7 (8×)); 0·945 d and 0·94 d (2 × CH_3, ^3J = 6·7).

XVIIe: ^{1}H NMR (D_2O): 8·92 s, 8·23 s, 7·96 bs, 7·49 bs, (4 arom. H); 3·47—4·56 m, 3 H (CH_2N, CH_2O, CH—O).

XVIIf: ^{1}H NMR (D_2O): 8·93 s, 8·36 s, 7·86 d, and 7·49 d (4 arom. H, ^3J = 1·5); 4·49 b^t (CH_2N, ^3J = 5·2); 3·99 bt (CH_2O, ^3J = 5·2); 3·55 d (O—CH_2—P, ^3J(H, P) = 8·4).

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