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Dedicated to Professor David Shugar on the occasion of his 80th birthday

Synthetic and biological applications of tricyclic analogues of guanosine

Bożenna Golankiewicz

Institute of Bioorganic Chemistry, Polish Academy of Sciences, Z. Noskowskiego 12/14, 61–704 Poznań, Poland

Key words: Y nucleosides, wyosine, tricyclic derivatives of guanosine and 2'-deoxyguanosine, *syn-anti* conformational analysis, 1D ^{1}H NOE differential spectroscopy, antiviral activity, acyclovir, ganciclovir

Tricyclic nucleosides incorporating the 9-oxo-imidazo[1,2-*a*]purine (1,N^2-ethenoguanine) system, natural prototypes of which occur in tRNA^{Phe} as nucleosides of the wyosine series, were used for synthetic, structural and biological purposes. 1,N^2-(Prop-1-ene-1,2-diyl)guanosine derivatives used as intermediates allowed to enforce on guanosine the substitution at the N-3 position and at the N^2 exo-amino group, not possible to be performed directly. Wyosine and 2'-deoxywyosine together with 4,5'-anhydro-4-desmethylwyosine and its congeners were used as, respectively, 100% *anti* and 100% *syn* conformation standards in a new graphical method for the *syn-anti* conformational analysis of nucleosides by 1D ^{1}H NOE difference spectroscopy. Substitution at the appended third ring allowed to modify the biological and physical properties of antiviral agents acyclovir and ganciclovir, e.g. to develop their fluorescent analogues.

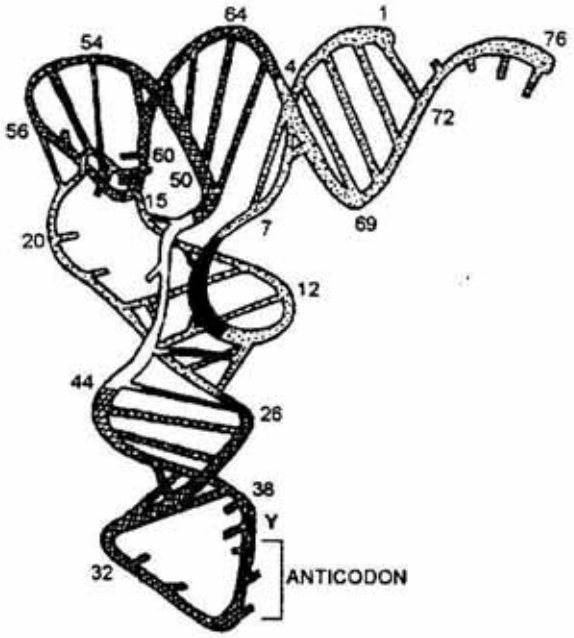
Among highly modified nucleosides characteristic for the anticodon region of transfer RNAs, the tricyclic nucleosides, called Y nucleosides or nucleosides of wyosine series, are some of the most distinctive. They occur at the position adjacent to the 3'-end of the anticodon triplet only in some tRNAs specific for phenylalanine within the tertiary structure which may be exemplified by that found from X-ray analysis of a single crystal of yeast tRNA^{Phe} (Scheme 1) [1].

The structure of Y nucleosides has been established as 4,9-dihydro-4,6-dimethyl-3-β-D-ribofuranosyl-9-oxoimidazo[1,2-*a*]purine which may additionally be substituted at the 7-position with a methyl group or a complex amino-acid chain [2–6]. These structures are shown in Scheme 2 together with the most recent symbols and common names for the particular members of the family [7].

Y nucleosides are strongly fluorescent and their glycosidic bond is very unstable under mild acidic conditions [2, 3, 8, 9], five orders of magnitude less stable than that of guanosine [10]. Y nucleosides are actually derivatives of guanosine: wyosine, the simplest representative of the series is 3-methyl-1,N^2-(prop-1-ene-1,2-diyl)guanosine.

Since their discovery in the late sixties [8] wyosine and its congeners have focused a considerable interest from the biological viewpoint [5, 11–16]. For a long time, their unusual site of methylation and the instability of the glycosidic bond have posed a challenge to chemical synthesis.

Abbreviations: ACV, acyclovir, 9-[(2-hydroxyethoxy)methyl]guanine; DHPG, ganciclovir, 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine; TK, thymidine kinase.



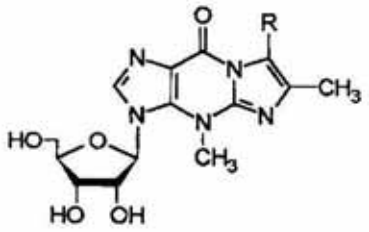
Scheme 1. Position of Y base within tertiary structure of transfer RNA^{Phe} (after [1]).

This review presents how the above tricyclic system occurring in a modified component of tRNA can be used for synthetic, structural and biological purposes.

Two groups have reported multistep synthesis of wyosine from 5-amino-1-(2′,3′,5-tri-*O*-acetyl-β-D-ribofuranosyl)-imidazole-4-carboxamide *via* 3-methylguanosine [10, 17–19]. After all modifications the overall yields were about 10%.

An entirely different approach was based on the results of biosynthetic studies. As it has been known since 1973 [20, 21] that Y nucleosides are biosynthetically derived from guanosine, an analogous chemical transformation was attempted (Scheme 3) [22].

Alkylation at the N-3 position has not been noted, even in traces, in any of the numerous papers on alkylation of guanosine and 2′-deoxyguanosine [22]. Therefore, guanosine to wyosine conversion *via* addition of the third ring to form 4-desmethylwyosine and subsequent methylation was approached. After testing a vast variety of methylation conditions which led either to the N-1 or to N-5 substituted products, it was found that only treatment of

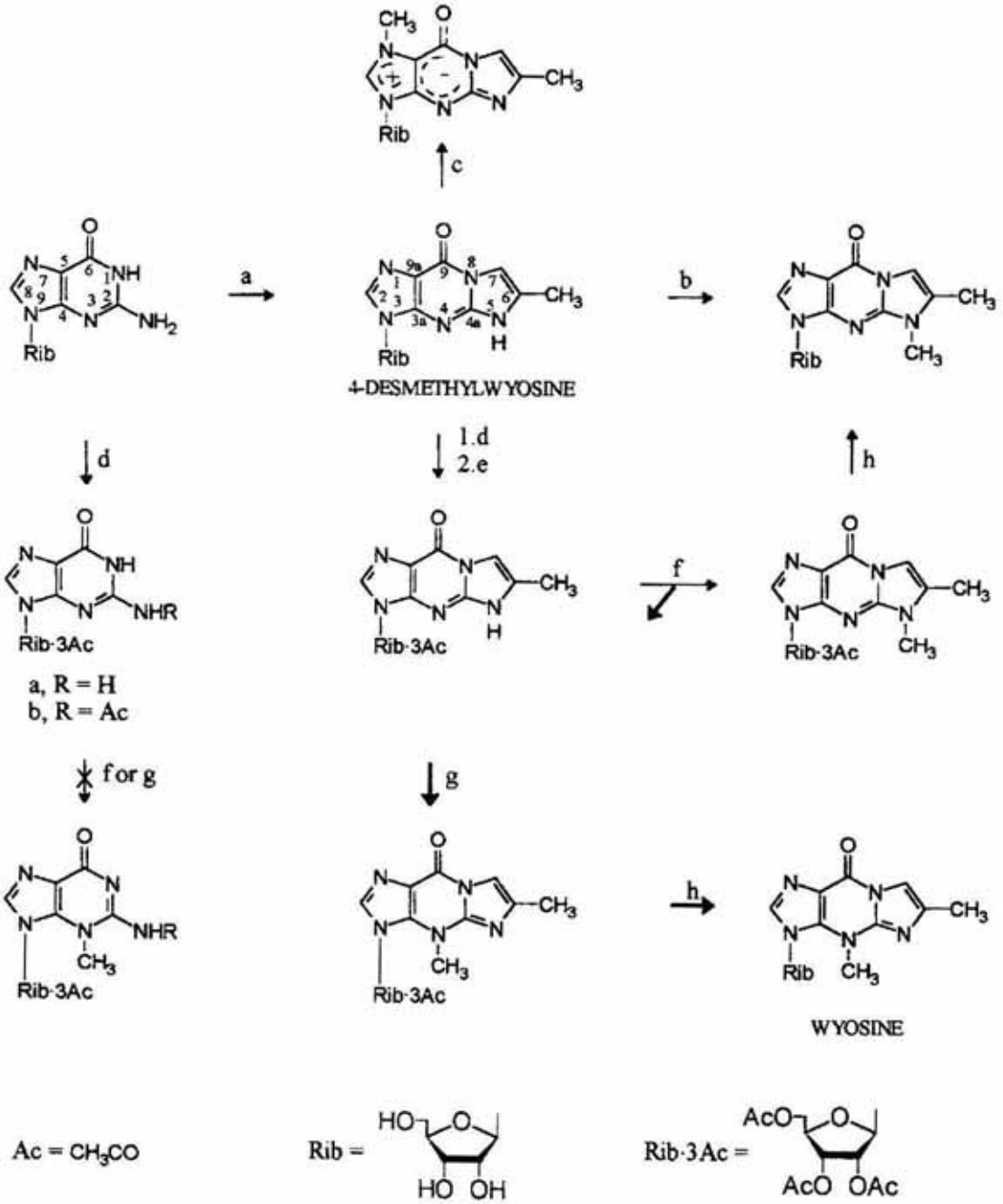


R	SYMBOL	COMMON NAME
H	imG	Wyosine
CH_3	mimG	Methylwyosine
$CH_2CH_2C(COOCH_3)(H)NHCOOCH_3$	yW	Wybutosine
$CH_2CH(OOH)C(COOCH_3)(H)NHCOOCH_3$	o_2yW	Peroxywybutosine
$CH_2CH(OH)C(COOCH_3)(H)NHCOOCH_3$	OHyW	Hydroxywybutosine
$CH_2CH(OH)C(COOH)(H)NH_2$	OHyW*	Undermodified hydroxywybutosine

Scheme 2. Structures, symbols and common names of Y nucleosides.

4-desmethylwyosine triacetate with diazomethane in methylene chloride gives the N-4 substituted product. However, it formed only a 3% admixture to the main, N-5 methylated product. The former was successfully separated by short column chromatography on silica gel and deblocked to give pure crystalline wyosine. This simple and inexpensive route represented the first procedure of wyosine synthesis described with complete experimental details [22].

Several years later it was found [23] that the yield of wyosine *via* methylation of 4-desmethylwyosine triacetate in methylene chloride could be improved to about 70% by applying Simmons-Smith "zinc reagent", broadly used before for cyclopropanation [24–27] but never for methylation. The reactions leading to vari-



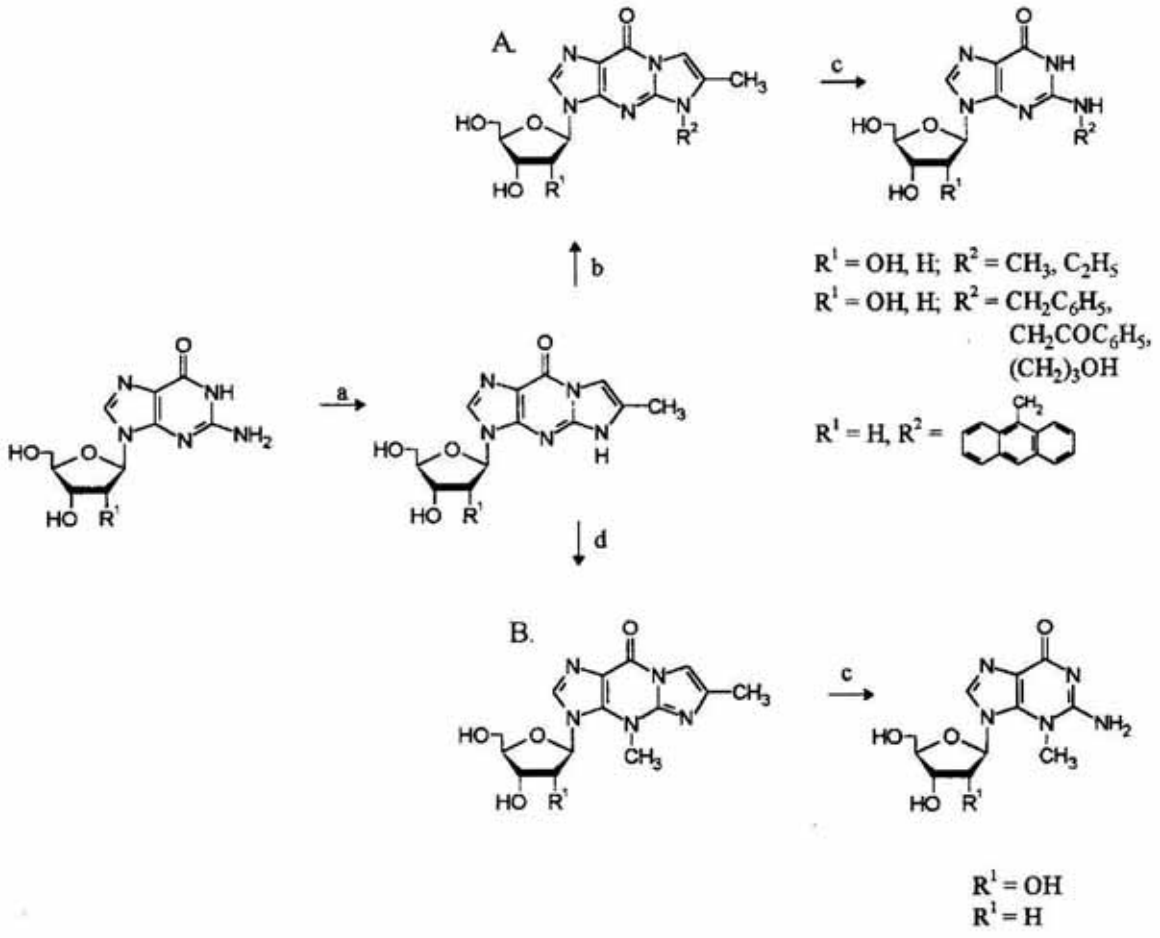
Scheme 3. Synthesis of wyosine from guanosine via direct N-4 methylation of the tricyclic 1,N^2-(prop-1-ene-1,2-diyl) derivative (4-desmethylwyosine) and other methylation products of 4-desmethylwyosine.
Reagents: (a) NaH/DMSO, $CH_2BrCOCH_3$; (b) K_2CO_3/DMF, CH_3I; (c) $(CH_3)_2SO_4$/DMF; (d) $(CH_3CO_2)O$, pyridine; (e) pyridine-CH_3OH-H_2O (1:1:1); (f) CH_2N_2/CH_2Cl_2; (g) $CH_2N_2/ZnI_2/Et_2O$ or $CH_2I_2/Zn(C_2H_5)_2/Et_2O$; (h) NH_3/MeOH.

ous methylation products of the tricyclic 4-desmethylwyosine are shown in Scheme 3.

The presence of the third ring is critical for the N-3 methylation to occur. All the attempts to methylate the N-3 position of guanosine triacetate or tetraacetate either with diazomethane/methylene chloride or with "zinc reagent" were unsuccessful (Scheme 3) ([28], Golankiewicz, B. & Ostrowski, T., unpublished results).

Appending of the third ring allowed to enforce on guanosine one more reaction not possible to be performed directly — alkylation of the exocyclic N^2 amino group. So far N^2 substituted guanosine derivatives have had to be obtained by tedious indirect approaches [29, 30]. As mentioned above, 4-desmethylwyosine (or using another name, 1,N^2-(prop-1-ene-1,2-diyl)guanosine) undergoes methylation at the N-5 position very readily. N-5 is the nitrogen which originally was the N^2 of the exoamino group of guanosine. It has been found that 1,N^2-(prop-1-ene-1,2-diyl) unit can be easily split off with *N*-bromosuccinimide to give high yields of various N^2-alkylated derivatives of guanosine and 2'-deoxyguanosine (Scheme 4A) [29]. The reaction was then used for preparation of a series of N^2-substituted guanosine derivatives of potential antiviral activity such as benzyl, benzoylmethyl, 3-hydroxytrimethylene [31]. That route was also applied to covalently bind 9-methylantracene to the N^2 exocyclic nitrogen of 2'-deoxyguanosine (Scheme 4A). The chemically synthesized modified unit was then incorporated into oligonucleotides in order to study the properties of double-stranded DNA containing this potentially carcinogenic lesion located in the minor groove [30].

The cleavage reaction using *N*-bromosuccinimide proved to be more general. It also worked



Scheme 4. Substitution at: (A), the N^2 exocyclic amino group and (B), the N-3 position of guanosine and 2'-deoxyguanosine mediated by the tricyclic derivatives.

Reagents: (a) NaH/DMSO, $CH_2BrCOCH_3$; (b) K_2CO_3 DMF, R^2X; (c) NBS, H_2O or buffer pH 4.8, then NH_3 aq.; (d) $(CH_3CO_2)O$, pyridine; pyridine-CH_3OH-H_2O (1:1:1); $CH_2N_2/ZnI_2/Et_2O$; NH_3/MeOH.

in the case of wyosine and 2'-deoxywyosine to give 3-methylguanosine and 3-methyl-2'-deoxyguanosine (Scheme 4B) [28, 32]. It worked despite the fact that electronic structures of the N-5 substituted or N-4, N-5 unsubstituted and N-4 substituted tricyclic analogues of guanosine are distinctly different. These differences are clearly shown by their ^{13}C and ^{15}N NMR spectra. For example the chemical shift differences are approx. 10 ppm for C-6 and C-3a [33], 90 ppm for N-5 and 65 ppm for N-4 [34].

3-Methylguanosine was obtained before by the aforementioned multistep synthesis [10, 17–19], whereas 3-methyl-2'-deoxyguanosine has not been obtained so far. Both compounds are interesting for several reasons.

They appear in RNA and DNA as a result of action of mutagenic methylation agents *in vitro* and *in vivo*. Their presence there has been inferred from the presence of 3-methylguanine in hydrolyzates [35, 36].

Methylation at N-3 changes hydrogen bonding characteristics of guanosine and 2'-deoxyguanosine. N-1 changes from a proton donor into a proton acceptor. That makes this pair of compounds similar to cytidine and 2'-deoxycytidine in their hydrogen bonding properties and may result in some mispairings along the RNA and DNA chains. Due to its changed hydrogen-bonding abilities, 3-methylguanosine may serve for probing the mechanism of action of enzymes using guanosine as substrate.

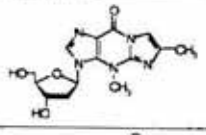
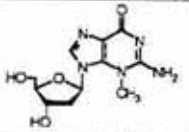
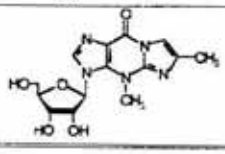
3-Methyl-2'-deoxyguanosine has the most labile glycosidic bond of all the nucleosides found in nature so far. As already mentioned, wyosine is five orders of magnitude less stable in that respect than guanosine. 3-Methyl-2'-deoxyguanosine is at least additional three orders of magnitude less stable. At pH 5.5 its half-life is 4 min whereas that of wyosine, considered so far as very unstable, is 19 days (Table 1) [32]. The data on the hydrolytic stability of 3-methyl-2'-deoxyguanosine built into DNA are of much interest. *In vivo* in *Escherichia coli* WP2 cells, its stability is comparable with that of monomer; *in vitro*, it is approx. 30 times higher (Table 1) [36].

The characteristic structural feature of wyosine, 3-methylguanosine and the corresponding 2'-deoxy congeners is the proximity of the sites bearing a sugar moiety and N-4 (N-3) methyl group. That is why the molecule seeks a nonstrained orientation of the sugar moiety relative to the base to alleviate steric hindrance. Such a conformation does exist: it is an almost perfect *anti*. Computer modeling based on force field calculation shows that wyosine and its congeners are fixed in that conformation. That property of wyosine was taken advantage of and used to develop a new NMR method of *syn-anti* conformational analysis [37].

The conformation about the glycosidic bond is one of the key parameters involved in the

Table 1

Apparent first-order rate constants (10^4k, s^{-1}) and half-lives (min) for the hydrolysis of the glycosidic bonds of 2'-deoxywyosine and 3-methyl-2'-deoxyguanosine at various pH values, 37°C.

	$k \times 10^4$, s^{-1}						
						in DNA	
pH	4.4	4.6	5.5	6.6	7.0	*in vitro* 7.2	*in vivo* in *E. coli*
	–	38.500 (3)	2.511 (46)	0.247 (468, 7.8 h)	0.055 (2100, 35.0 h)	–	–
	–	–	28.875 (4)	1.179 (98)	0.722 (160, 2.6 h)	(105 h)	(3.6 h)
	0.051 (37.5 h)	–	0.004 (19 days)	–	–	–	–

structure of oligo- and polynucleotides, as well as in the interaction of nucleosides and nucleotides with many enzymes [38, 39].

The *syn-anti* conformation of nucleosides has been extensively studied using various ^{1}H and ^{13}C NMR techniques but there are still discrepancies between the data obtained by various methods.

A new semiquantitative method for estimation of the contribution of *syn* and *anti* population to conformation of nucleosides [37] follows the former approach [38, 39] in using model compounds for 100% *anti* and 100% *syn* conformation but, instead of analysis of chemical shifts of H-2' and C-2', it uses one dimension proton nuclear Overhauser enhancement (1D ^{1}H NOE) difference spectroscopy. It is a graphical method based on the data for conformationally rigid molecules as calibration points — in *anti* mode wyosine and 2'-deoxywyosine, in *syn* 3,5'-anhydroisoguanosine. The diagnostic proton is that at C-8 in purine nucleosides and at C-6 in pyrimidine nucleosides. In the case of an *anti* compound it is closer to 2' and 3' protons and farther from 1'proton; in the case of a *syn* compound the reverse is true. Perfect *anti* and perfect *syn* structures of the model compounds found from computer modeling are reflected by NOE values — upon irradiation of H-8 (H-2 in the numeration system of wyosine) they are 9.5% for the sum of 2' and 3' protons in wyosine and 2'-deoxywyosine and 11.3% for 1' proton in 3,5'-anhydroisoguanosine. Having measured the 1D ^{1}H NOE spectra of nucleosides one can read from the graph (Fig. 1) the conformational preferences. For example in adenosine (1β) *syn* conformation prevails (60% *syn*) while guanosine (19β) exhibits *anti* orientation of the nucleobase (70% *anti*). More than 50 regular and modified nucleosides were analyzed in this way by Rosemeyer *et al.* [37].

The structural differences between wyosine and 3,5'-anhydroisoguanosine may raise some doubts about their reliability as standards. That is why further "100% *syn*" standards with high structural resemblance to wyosine: 4,5'-anhydro-4-desmethylwyosine and its 2'-deoxy and 2',3'-dideoxy congeners were synthesized and tested. The NOE value on H-1' after irradiation of H-2 had a constant value of 11.0 ± 0.1%, very similar to that found for 3,5'-anhydroisoguanosine, which demonstrated that all these anhydronucleosides could serve as general standards for 100% *syn* conformation (Scheme 5) (Golankiewicz, B., Zeidler, J., Rosemeyer, H. & Seela, F., unpublished).

The tricyclic derivatives also proved to be of use in the studies on potential antivirals from the family of acyclonucleosides.

Acyclonucleosides, the analogues in which the sugar moiety of a molecule has been replaced with an aliphatic chain mimicking the fragment of the sugar, have focused much attention since the discovery of potent and selective antiherpetic activity of an acyclic analogue of guanosine, 9-[(2-hydroxyethoxy)methyl]-guanine [40]. This analogue known as acyclovir (Scheme 6) is perhaps the least toxic of antiviral agents known so far. Acyclovir owes its antiherpetic selectivity to specific phosphorylation by the virus encoded deoxythymidine kinase which confines further action to the virus-infected cell. The resulting acyclovir monophosphate is further phosphorylated to the triphosphate form by cellular kinases. Acyclovir triphosphate interferes with viral DNA synthesis through both a direct inhibitory effect on the viral DNA polymerase and a chain terminating effect (following incorporation at the 3'-end) [40, 41].

Modifications of the acyclic side chain of acyclovir have given rise to several compounds with significant selective antiviral activity [41, 42]. Some of them which have been marketed as antiherpetic drugs are shown in Scheme 6. Of these 9-[(1,3-dihydroxy-2-propoxy)me-

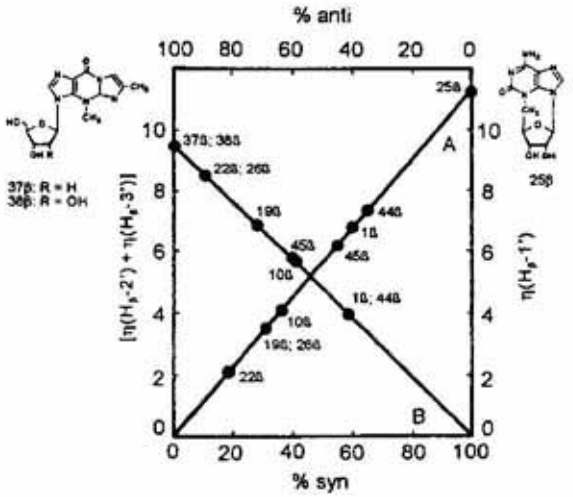
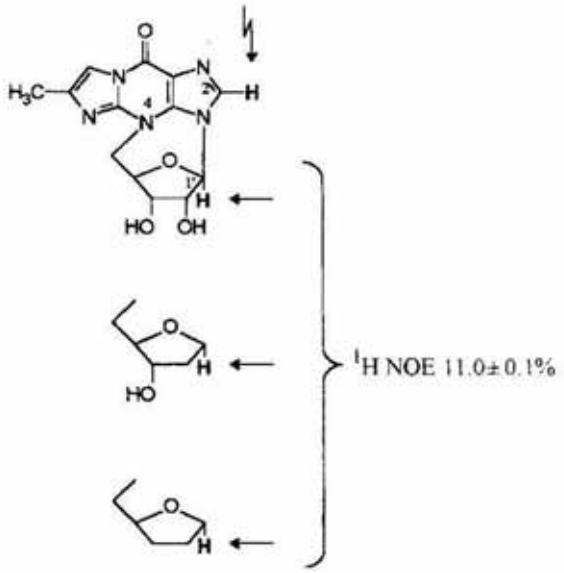


Fig. 1. Calibration graph for the estimation of syn and anti conformer populations of β-D-*nucleosides applying* ^{1}H *NOE difference spectroscopy (after [37]).*



Scheme 5. Structure of 4,5'-anhydro-4-desmethylwyosine and its 2'-deoxy and 2',3'-dideoxy congeners, the tricyclic, alternative "100% syn" standards for the graphical method shown in Fig. 1.

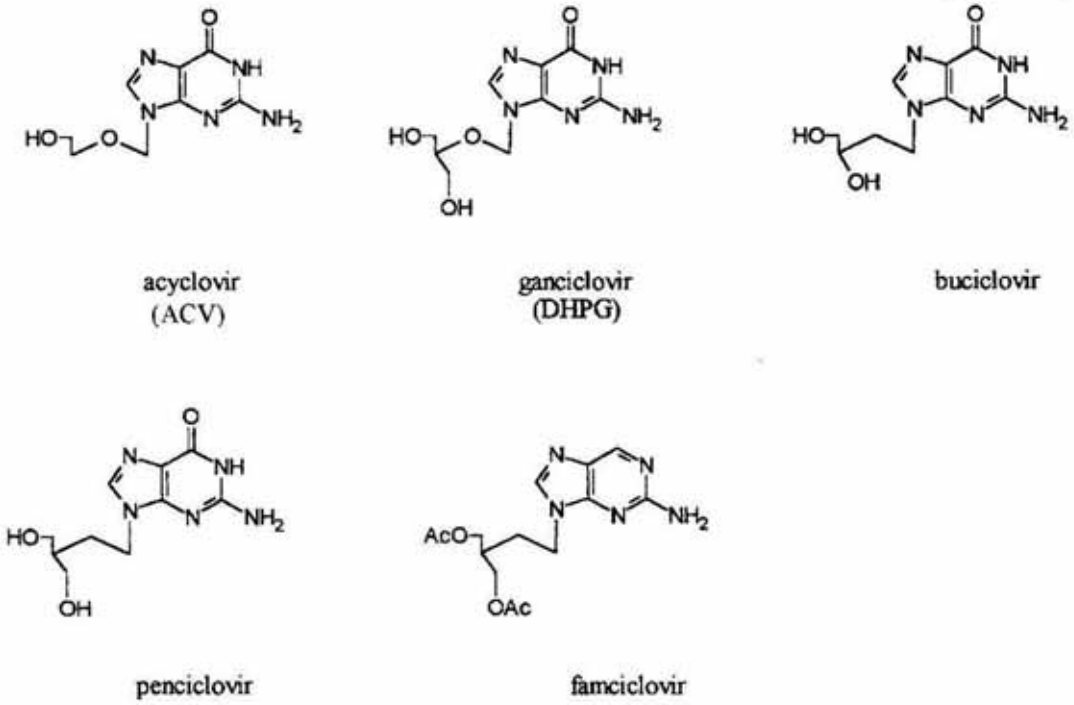
thyl]guanine, known as ganciclovir or DHPG has been the one most extensively studied for its antiviral properties.

On the contrary, modifications of the guanine moiety of acyclovir have been reported so far as virtually annihilating the antiviral activity of acyclovir [43].

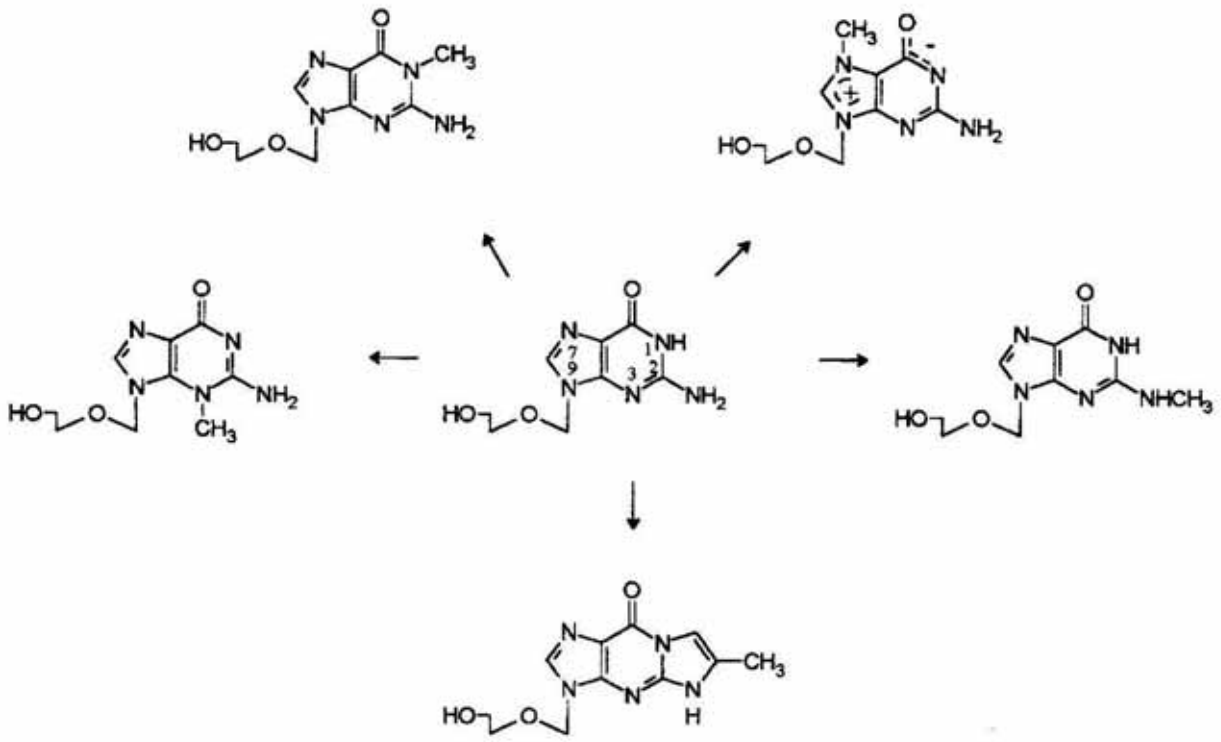
In search to delineate the structural features of the base moiety of acyclovir which are crucial for its antiviral activity it was decided to assess the importance of the nitrogen centers. In this perspective several new acyclovir derivatives in which one or more nitrogen centers were blocked by methylation or incorporation into an additional ring were prepared (Scheme 7) [44, 45].

All the procedures for direct and indirect substitution of particular nitrogen centers, discussed above for regular nucleosides (Schemes 3, 4), worked also in the acyclo series.

The newly synthesized N-substituted derivatives of acyclovir were examined for their inhibitory effects on the replication of a wide variety of DNA viruses including herpes simplex virus type 1 (HSV-1) (strains KOS, F, McIntyre), herpes simplex virus type (HSV-2) (strains G, 196, Lyons), thymidine kinase deficient (TK$^-$) HSV-1 mutants (B2006, VMW 1837), varicella-zoster virus (VZV) (strains YS, OKA), TK$^-$VZV mutants (YSR, 07-01), and cytomegalovirus (CMV) (strains Davis, AD-169). It was found that methylation at N-3 and at N^2-amino group annihilated the antiviral activity of acyclovir, whereas methylation at N-7 only reduced it. Position N-1 did not appear important



Scheme 6. Examples of acyclonucleosides with selective antiviral activity.



Scheme 7. Acyclovir derivatives with blocked nitrogen centers.
The order of importance of nitrogen centers in the antiviral activity of acyclovir N-3 ≥ N^2 > N-7 > N-1.

in this respect, since 1-methylacyclovir showed considerable antiviral activity. The N-3 position must play a significant role in the biological activity of acyclovir. In 3-methylacyclovir two nitrogen centers, exocyclic N^2- amino and N-7, crucial for antiviral activity, were conserved. Yet, the compound was inactive. In summary, the antiviral data indicate the following order of decreasing importance in the antiviral activity of acyclovir: N-3 ≥ N^2 > N-7 > N-1 [44, 45].

As in the tricyclic 1,N^2-(prop-1-ene-1,2-diyl)acyclovir {according to the IUPAC systematic nomenclature 3,9-dihydro-3-[(2-hydroxyethoxy)methyl]-6-methyl-9-oxo-5*H*-imidazo-[1,2-*a*]purine} the exocyclic NH_2 group at C-2 is blocked, the compound could have been expected to be inactive. That was not the case, the tricyclic analogue exhibited a potent and selective antiherpetic activity. The spectrum of its activity was narrower than that of acyclovir, limited to HSV-1 and HSV-2; lower cytoxicity resulted in a higher selectivity index than that of acyclovir itself [44].

The studies were therefore extended to additional tricyclic analogues bearing either a 3*-[(1,3-dihydroxy-2-propoxy)methyl] side chain — that is ganciclovir analogues or a 6*-unsubstituted appended imidazole ring, or both [46]. It was found that the enhancement of activity with introduction of (1,3-dihydroxy-2-propoxy)methyl residue was higher than that for the pair acyclovir-ganciclovir. Besides, the 6-methyl substituent appeared to be of importance: its absence resulted in 6–1000 fold decrease of antiviral activity, which implied that substitution might become a way to shape the physical and biological properties of the tricyclic analogues of acyclovir and ganciclovir.

Along this line the effect of substitution in the imidazopurine moiety of these analogues on their physical and antiherpetic activity was investigated by synthesizing a series of compounds substituted in the 2, 6 or 7 position. Substitution in the 6-position with phenyl of 4-biphenylyl resulted in fluorescent compounds [47].

*Throughout the discussion the numbering of the positions of tricyclic compounds is in the systematic IUPAC convention.

The most interesting results concerning the structure-activity relationship are presented in Table 2.

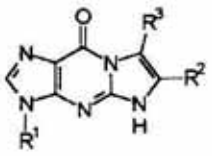
The decrease of antiherpetic activity after linking the 1 and N^2 positions of guanine moiety of acyclovir with an etheno bridge ranges, depending on the type and strain of the virus, from 100 to over 2000 fold. Further substitutions in the resulting ring enhance the antiviral activity. The magnitude of the antiviral effect depends upon (i) the position and the type of the substituent, (ii) the nature of the virus, and (iii) the kind of the acyclic moiety in the 3 position of the heterocycle. For example, the increase of activity following introduction of methyl group in either 6 or 7 position for HSV-2 strains is comparable; for VZV the 6-methyl compound is 6–10 fold more active than 7-methyl. Substitution of $1,N^2$-ethenoacyclovir with 6-methyl group gives a compound at least 30 times less active than the parent acyclovir; in the case of ganciclovir, the 6-methyl-$1,N^2$-etheno derivative is as active against certain strains as the parent compound.

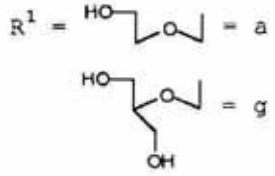
Of the different molecules presented here the fluorescent 3,9-dihydro-3-[(1,3-dihydroxy-2-propoxy)methyl]-9-oxo-6-phenyl-5*H*-imidazo-[1,2-*a*]purine is the most promising. Its activity against HSV-1, HSV-2, TK^-HSV-1, TK^+VZV and TK^-VZV is very similar to that of the parent ganciclovir, only its activity against CMV is one order of magnitude lower. This compound may prove useful in the noninvasive diagnosis of herpes virus infection. Because of its fluorescence the compound and its metabolites could be monitored as "tags" for the virus-infected cells and virus-specified enzymes [47].

To summarize, the tricyclic modification of guanosine and its congeners, inspired by the structure of nucleosides occurring in $tRNA^{Phe}$, allowed to develop: (i) synthetically valuable new reactions of guanosine, (ii) 1D ^1H NOE graphical method for the *syn-anti* conformational analysis based on conformationally rigid

Table 2

Activity against human herpesviruses and cytotoxicity of selected tricyclic analogues of acyclovir and ganciclovir





minimal inhibitory concentration μg/mL

Virus (strain)	R^1 R^2 R^3	ACV acy-clovir	a H H	a Me H	a H Me	a *t*-Bu H	a Ph H	GCV ganci-clovir	g H H	g Me H	g Ph H
HSV-1 (KOS)		0.02	20	0.8	2.1	4.5	0.4	0.003	0.7	0.015	0.02
HSV-1 (F)		0.004	4	0.58	0.1	4.0	0.7	0.004	0.7	0.005	0.005
HSV-2 (G)		0.09	20	3	4.5	13	1.3	0.02	2.0	0.1	0.3
HSV-2 (196)		0.01	70	1.5	2	7	0.2	0.04	2.0	0.2	0.02
VZV (YS)		0.38	> 400	9.4	> 50	2.2	1.4	1.4	> 400	12	3
VZV (OKA)		0.18	> 400	4.6	> 50	1	1.3	0.5	400	1	0.4
TK^- VZV (YS-R)		4.7	> 400	70	> 50	127	67	1.4	300	15	1
CMV (Davis)		ND	> 400	> 50	> 50	< 400>100	166	0.9	200	> 50	7
Morphological alteration		350	> 400	> 190	> 100	> 250	> 100	> 270	> 400	> 175	> 175
Cell growth		> 200	> 200	> 200	> 200	167	45	> 200	> 200	> 200	> 200

molecules, (iii) fluorescent analogues of the antiviral drugs acyclovir and ganciclovir.

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