
Carbocyclic Nucleosides

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I. INTRODUCTION

The term carbocyclic nucleoside is used to describe a group of compounds structurally analogous to natural and synthetic nucleosides in which the furanose oxygen has been replaced by a methylene group. This transformation, therefore, changes the furanose ring into a cyclopentane. It is perhaps more

correct to refer to these compounds as *carbocyclic nucleoside isosteres* because, strictly speaking, they are not nucleosides. Carbocyclic nucleosides, however, is a convenient term because these compounds undoubtedly exert their biological activity by mimicking the parent nucleosides. Consistent with the presence of the carbocyclic ring they are not subject to the action of nucleoside phosphorylases and hydrolases that cleave normal nucleosides. Conformationally, however, the expected similarity in bond lengths and bond angles between the tetrahydrofuran and cyclopentane rings allow these analogues to behave as substrates or inhibitors of the enzymes that activate and interconvert nucleosides and nucleotides in living cells. As a result of this likeness, many of these compounds are endowed with an interesting range of biological activities, especially in the areas of antiviral and anticancer chemotherapy. The majority of carbocyclic nucleosides known to date are of synthetic origin but nature has provided two of the most active compounds, aristeromycin (**35a**) and neplanocin-A (**83**). Although, to our knowledge, no comprehensive review on carbocyclic nucleosides has appeared in the literature, several publications have addressed this topic as a subcategory in sufficient detail to be considered as short reviews.¹⁻³

The present review will encompass a discussion of both the chemistry and the biological properties of this unique group of compounds with special emphasis on those in which the structure resembles more closely that of conventional nucleosides.

A word of caution in relation to the nomenclature of these compounds is appropriate at this point. Throughout the literature, carbocyclic nucleosides are identified with a capital letter "C" followed by the name, or abbreviated name, of the corresponding nucleoside isostere (i.e., C-adenosine or C-Ado). This notation will be used throughout this article and ought not to be confused with that describing carbon nucleosides which, as a group, are referred to as C-nucleosides. In addition, carbocyclic nucleosides will be identified in this article with the same numbering system used in the description of their nucleoside isosteres.

II. THE CHEMISTRY OF CARBOCYCLIC NUCLEOSIDES

A. General Discussion

Several carbocyclic nucleosides were conceived and synthesized by medicinal chemists prior to the isolation of the carbocyclic adenosine prototype

Victor E. Marquez was born in 1943 in Caracas, Venezuela. He received a B.S. in Pharmacy from the Universidad Central de Venezuela in 1966 and later in 1970 a Ph.D. in Medicinal Chemistry from the University of Michigan. After spending 1 year as a postdoctoral fellow at the National Cancer Institute, NIH, and 5 years as Director of Research of Laboratorios Cosmos in Caracas, he returned to the NCI to his present position of Visiting Scientist. His current research interests involve the design and synthesis of nucleoside antitumor drugs based on mechanistic considerations. He has published over 40 research articles and has 5 patents.

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aristeromycin from natural sources.⁴⁻¹² Some of the initially synthesized compounds were simple cyclopentyl substituted bases,^{11,12} but others included true isosteres of thymidine (C-Thy, **57b**) and adenosine (C-Ado, **35a**, or (\pm)-aristeromycin).^{9,10} The first reported synthesis of C-Thy,⁸ however, was found to be in error but the correct compound was later prepared.¹³⁻¹⁵ Most current synthetic approaches begin with the construction of the base from a functionalized cyclopentylamine (*vide infra*) which with very few exceptions is obtained as a racemic mixture. Consequently, most of the reported synthetic carbocyclic nucleosides are racemates. Recently, however, a long overdue enantioselective synthesis of aristeromycin has been finally achieved^{16,17} and of the three total synthesis of neplanocin A reported in 1983, two are enantioselective.¹⁶⁻¹⁹

B. Synthesis of Carbocyclic Ribofuranosylamine (C-rib-NH₂)

The basic strategy in the synthesis of carbocyclic nucleosides has remained substantially unchanged since Shealy's original work.^{9,10} It involved: (1) synthesis of the carbocyclic ribofuranosylamine **6** (C-rib-NH₂, Figure 1), and (2) construction of the purine or pyrimidine ring from this amine by well established procedures in nucleoside chemistry. The other syntheses that followed differed mainly in the novelty and efficiency of producing the desired C-rib-NH₂ with the correct stereochemical disposition of substituents. Figure 1

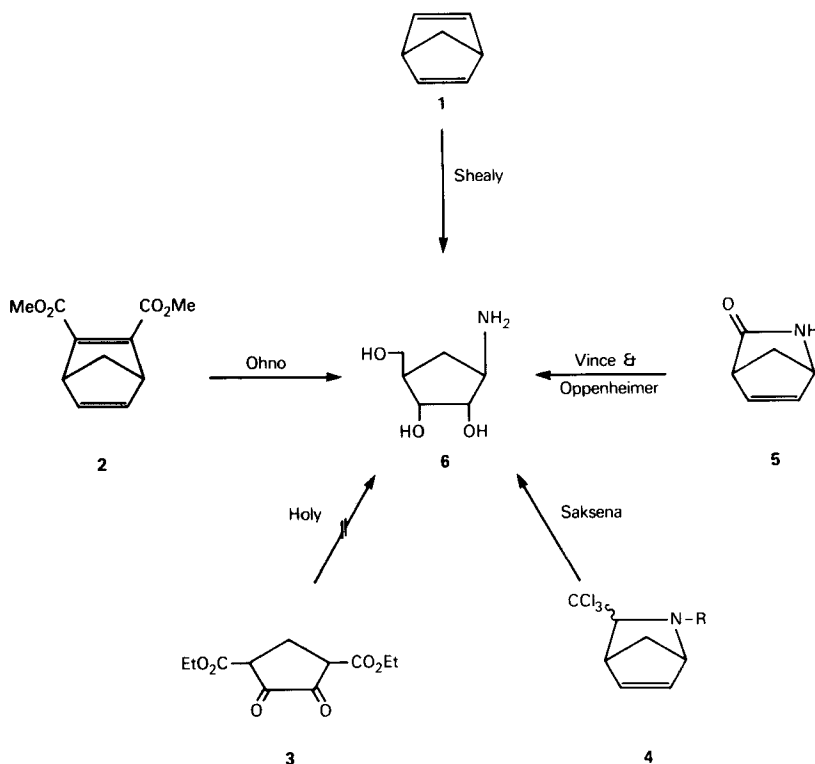
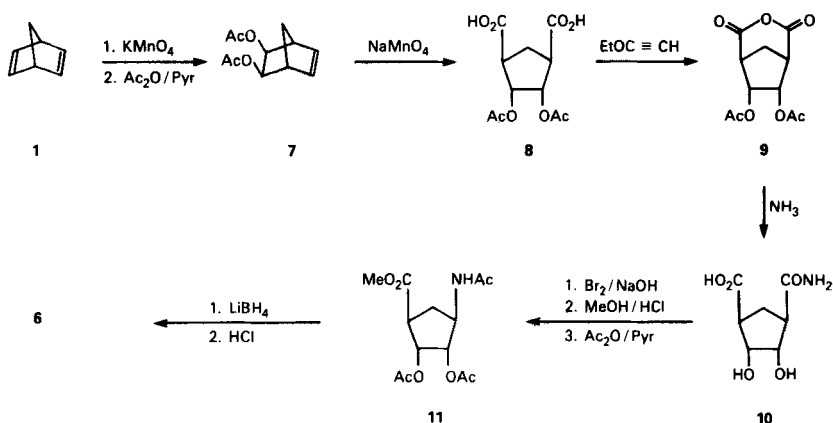
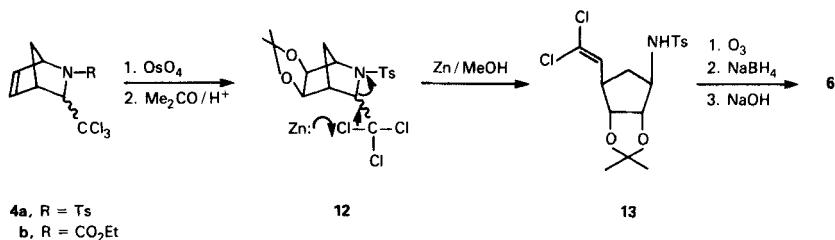


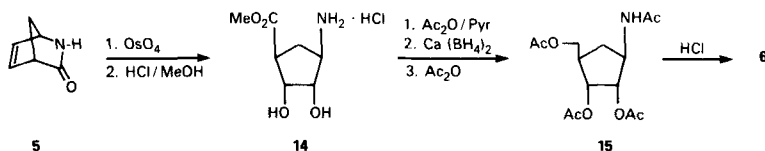
Figure 1. Synopsis of the different approaches to C-rib-NH₂.



depicts a summary of the five different approaches to C-rib-NH₂ that have been reported thus far.^{9,10,17,20-24}

With the exception of Holy's approach,^{20,21} all the syntheses utilized a rigid bicyclo[2.2.1]heptene system which allowed for better control of the stereochemistry of incoming substituents in subsequent reactions. When norbornadienes **1** and **2** were used as starting materials, the extra carbon atom in the molecule was replaced by the required amino function via a Hoffman rearrangement of a carboxylic acid amide generated after ozonolysis of one of the double bonds. Later, in an effort to overcome the use of the Hoffmann reaction, azabicyclo[2.2.1]heptene systems **4** and **5**, which already contain a latent amine functionality, permitted a more efficient generation of C-rib-NH₂ (Vince's and Saksena's methods).²²⁻²⁴ Use of an enzyme in the hydrolysis of *meso* diester **2** yielded an optically active half-ester from which the enantiomerically pure C-ribo-NH₂ was obtained in Ohno's synthesis.¹⁷ Conversely, lack of a reagent capable of discriminating between symmetric groups in Shealy's method led to the desired amine only in its racemic form.^{9,10} Finally, in Holy's approach, an attempt was made to convert cyclopentanedione **3** to racemic C-rib-NH₂.^{20,21} It is questionable, however, that the correct compound was obtained due to the differences between a putative C-Ado prepared from **3**, and samples of authentic (-)-aristeromycin and synthetic C-Ado (**35a**), obtained by other methods.²⁵ A brief discussion of these syntheses follows: Cis-hydroxylation of 2,5-norbornadiene **1** followed by acetylation occurred stereospecifically producing the expected *exo* adduct diacetate **7**. Oxidative



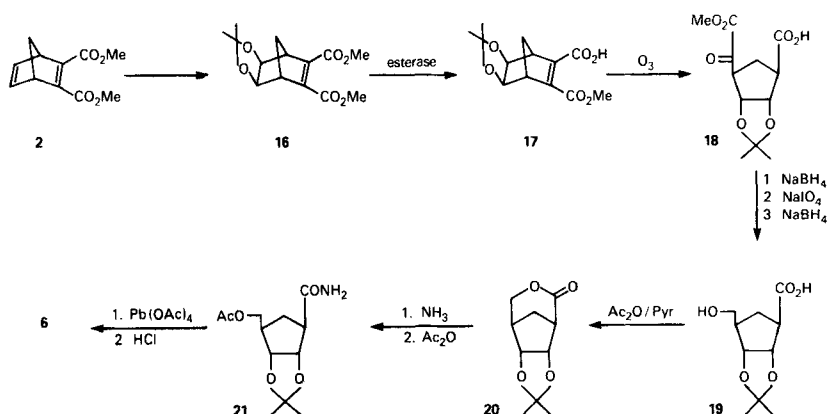


Scheme III
Vince and Oppenheimer^{23,24}

cleavage of the double bond led to diacid **8**, which was dehydrated to the anhydride **9**. After opening the anhydride with ammonia, the resulting half amide **10** was subjected to the Hoffmann reaction, esterification, and reacylation to give the triacetate **11**. From the opening of the anhydride **9** an enantiomeric pair was generated and therefore all cyclopentyl derivatives obtained beyond this point are racemic (only one enantiomer is depicted). Reduction of the methyl ester and removal of the acetyl groups resulted in the formation of racemic C-ribo-NH₂(**6**).

In 1980 Saksena reported the synthesis of (±)C-Ado starting with racemic 2-azabicyclo[2.2.1]heptenes **4a** and **4b** which had been previously obtained in excellent yields.^{21,22} After catalytic osmylation of **4a** and formation of the acetone **12**, a key reductive ring opening with activated zinc afforded alkene **13**. Sequential ozonolysis, reduction, and hydrolysis, afforded the isopropylidene derivative of the target amine **6**. The yields were inferior when **4b** was used as the starting material.

The stable (±)-2-azabicyclo[2.2.1]hept-5-ene-3-one (**5**) prepared earlier by Jagt and Van Leusen²⁶ was used in several instances by Vince and Daluge since 1976 to prepare various carbocyclic nucleosides and aminonucleosides.²⁷⁻³⁰ However, it was only in 1981 that they reported the direct osmylation of **5** which gave stereoselectively compound **14** after methanolysis of the lactam ring.²³ When osmylation was performed earlier on the ring-opened hydrolyzed product of **5**, regioselectivity was lost and a mixture of isomers was produced.³⁰ From **14**, acetylation, reduction, and reacylation, gave the tetraacetate **15** from which racemic C-rib-NH₂ (**6**) was obtained. In the same year Oppenheimer and Kam



Scheme IV
Ohno et al.^{16,17}

independently reported a nearly identical procedure which employed KMnO_4 as an efficient *cis*-hydroxylating reagent.²⁴

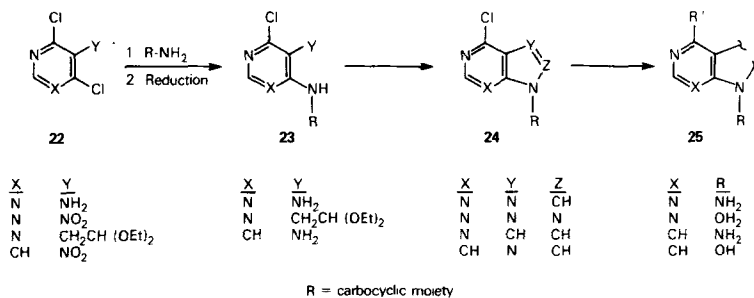
In a similar fashion as performed for the enantioselective synthesis of C- and N-nucleosides, which started with a non-carbohydrate moiety,³¹ Ohno's group succeeded in producing optically active C-ribo- NH_2 . Pig liver esterase asymmetrically hydrolyzed the known mesodiester **16**,³² to give the asymmetric acid **17**. This acid was transformed into ketoacid **18** after a decarboxylative ozonolysis; and, following the successive treatment of **18** in a sequence which involved borohydride reduction, periodate cleavage of the vicinal glycol, and a second borohydride reduction, the hydroxyacid **19** was obtained. Lactonization of **19** followed by lactone opening with ammonia and acetylation, afforded amide **21**. This compound was subjected to a Hoffmann reaction with $\text{Pb}(\text{OAc})_4$ which led to the target cyclopentylamine **6** after removal of the protective groups. The absolute configuration of **6** was shown to be identical to that of ribose as demonstrated by the fact that natural (-)-aristeromycin was obtained from it.

C. General Methods of Aglycone Synthesis

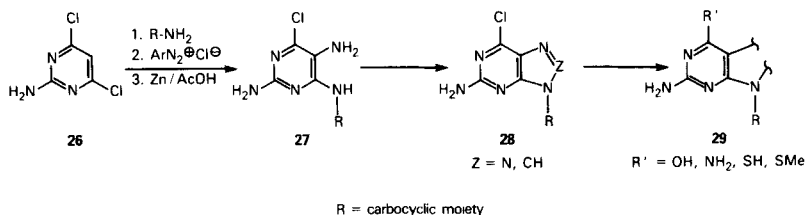
Among the various synthetic approaches to purines and pyrimidines only a few methods have been used in carbocyclic nucleoside chemistry, mainly because of the early commitment to the synthesis via the cyclopentylamine.

a. Purines

The time-honored route inspired in the Traube synthesis³³ has been used to convert the carbocyclic amine (R-NH_2) to the corresponding pyrimidylaminocyclopentane derivative **23** which is then followed by completion of the pyrrole, imidazole, or triazole ring, to give the corresponding purine carbocyclic nucleoside **24** (Scheme V). The reactive 6-chloro substituent allows replacement with ammonia or water to give the adenine and hypoxanthine analogues (**25**), respectively. Completion of the bicyclic system varies accordingly: it consists of (1) a spontaneous acid-catalyzed cyclization from **23** ($\text{X} = \text{N}$, $\text{Y} = \text{CH}_2\text{CH}(\text{OEt})_2$) in the case of the 7-deazapurine system; (2) formation of the imidazole ring from **23** ($\text{X} = \text{N}$, or $\text{X} = \text{CH}$, $\text{Y} = \text{NH}_2$) after treatment with an activated one-carbon reagent (e.g. triethylorthoformate); or, (3) diazotization of the primary aromatic amine in **23** ($\text{X} = \text{N}$, $\text{Y} = \text{NH}_2$) to give the



Scheme V



Scheme VI

8-azapurine analogue. The compounds prepared by these procedures are listed in Table I.

The carbocyclic guanine and 2,6-diaminopurine analogues were obtained similarly as shown in Scheme VI. In this instance, formation of the pyrimidylaminocyclopentane derivative was followed by a phenyldiazo insertion and reduction with zinc, to afford the additional amino group in **27** necessary for the completion of the bicyclic system.

b. Pyrimidines

With the exception of first attempt to prepare the thymidine analogue (C-Thy, **57b**)⁸ which was later found to have given an isomeric product distinct from C-Thy,¹⁵ all reported syntheses of carbocyclic pyrimidines have made use of preformed carbocyclic amines as starting materials. The procedures apply the general methodology for the synthesis of uracil and thymine as developed earlier by Shaw and Warrener.³⁴⁻³⁶ Isocyanate **30** ($R' = Me$ or Et , $X = H$ or Me) was reacted with carbocyclic amine ($R-NH_2$) to give an intermediate acryloylurea **32** which was then cyclized in the presence of concentrated ammonia, or with acid catalysts, to give the uracil (**33**, $X = H$) or thymine (**33**, $X = CH_3$) analogue (Scheme VII). Alternatively, the same result was obtained by reacting the carbocyclic amine with 3-ethoxy-N,2-bis(ethoxycarbonyl)acrylamide (**31**) to give the 5-carboethoxyuracil (**33**, $X = CO_2Et$). This substituent was later removed by hydrolysis and decarboxylation. The generated unsubstituted carbocyclic uridine derivatives (**33**, $X = H$) were amenable to direct halogenation at C-5 and the halogen later displaced by a host of nucleophiles to produce a number of 5-substituted uridine analogues.

Transformation of the uracil ring into cytosine required conversion of the cyclic amide in **33** to the 4-chloropyrimidine (**34**, $X = Cl$) which reacted with ammonia to give **34** ($X = NH_2$). Alternatively, thiation of the uridine analogue **33** to the corresponding 4-thiouracil derivative, followed by methylation and ammonolysis, produced identical results. The reported carbocyclic uridine and cytidine derivatives are listed in Table II.

III. Purine Carbocyclic Nucleosides.

A. Purine Carbocyclic Nucleosides.

This group includes compounds with an intact imidazo[4,5-d]pyrimidine (purine) ring system bearing different 9-cyclopentyl substituents that mimic the several known sugar moieties of the corresponding nucleoside counter-

Table I.
Carbocyclic Purine Nucleosides

<u>S</u>	<u>Compound No.</u>	<u>X</u>	<u>Y</u>	<u>Z</u>	<u>Ref.</u>
	35a (C-Ado, aristeromycin)	NH ₂	H	H	4-6,9,10,16,17,22
	b	Cl	H	H	9,10,37
	c	NHMe	H	H	37
	d (C-Ino)	OH	H	H	10,37,38
	e	OMe	H	H	37
	f	NHOH	H	H	37
	g	SH	H	H	10,37,38
	h	SMe	H	H	10,38
	i	NH ₂	NH ₂	H	39
	j	Cl	NH ₂	H	39
	k	NHMe	NH ₂	H	39
	l (C-Gua)	OH	NH ₂	H	39,40
	m	SH	NH ₂	H	39
	n	SMe	NH ₂	H	39
o	NH ₂	H	Br	38	
p	NH ₂	H	OH	38	
	36a	NH ₂	H	H	27,29
	b	NMe ₂	H	H	27,29
	37a	NH ₂	H	H	38,41
	b	Cl	H	H	41
	c	Cl	NH ₂	H	42
	d	OH	NH ₂	H	42
	e	SH	NH ₂	H	42
	f	NH ₂	NH ₂	H	42
	38a	NH ₂	H	H	38,41
	b	Cl	H	H	41
	39a (C-ara-A)	NH ₂	H	H	28
	b	Cl	H	H	28
	c	SH	H	H	43
	d	SMe	H	H	43
	e	NMe ₂	H	H	43
	f	NH ₂	NH ₂	H	44
	g (C-ara-Gua)	OH	NH ₂	H	44
	h	Cl	NH ₂	H	44
	40	NH ₂	H	H	27,29
	41a	NH ₂	H	H	45
	b	Cl	H	H	45
	42a	NH ₂	H	H	30
	b	Cl	H	H	30
	43a	Cl	H	H	12
	b	OH	H	H	12
	c	SH	H	H	12
	d	NH ₂	H	H	12
	e	NHMe	H	H	12
	f	NMe ₂	H	H	12

Table I.
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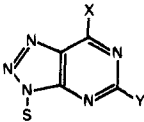
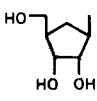
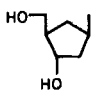
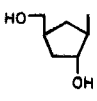
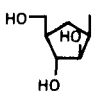
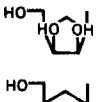
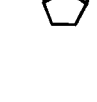
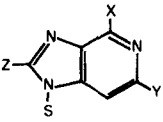
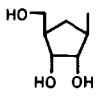
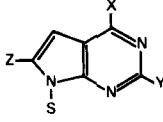
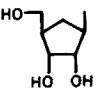
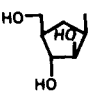
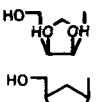

					
	44a (<i>β</i> - <i>aza</i> -C-Ado)	NH ₂	H		46,47
	b (<i>β</i> - <i>aza</i> -C-Ino)	OH	H		47
	c	NH ₂	NH ₂		39
	d	Cl	NH ₂		39,40
	e	SMe	NH ₂		39
	f (<i>β</i> - <i>aza</i> -C-Gua)	OH	NH ₂		39,40
	45a	NH ₂	H		47
	b	OH	H		47
	c	Cl	NH ₂		42
	d	OH	NH ₂		42
	e	NH ₂	NH ₂		42
	46a	NH ₂	H		47
	b	OH	H		47
	47a	OH	NH ₂		44
	b	NH ₂	NH ₂		44
	c	Cl	NH ₂		44
	48a	NH ₂	H		30
	b	OH	H		30
	48a	NH ₂	H		47
	b	OH	H		47
					
	50a	NH ₂	H	H	48
	b	Cl	H	H	48
	c	NH ₂ NH ₂	H	H	48
	d	NH ₂	H	H	48
					
	51a	NH ₂	H	H	49
	b	Cl	H	H	49
	c	NH ₂	H	Me	50
	d	Cl	H	Me	50
	52a	NH ₂	H	Me	50,51
	b	Cl	H	Me	50,51
	c	-N ₅ CH ₂ CH ₂ CH ₂	H	Me	51
	d	-N ₅ CH ₂ CH ₂ CH ₂ CH ₂	H	Me	51
	53a	NH ₂	H	Me	50,51
	b	Cl	H	Me	50,51
	54	NH ₂	H	H	52

Table I.
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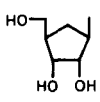
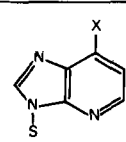
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Table II
Carbocyclic Pyrimidine Nucleosides

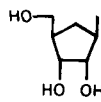
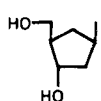
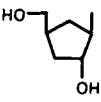
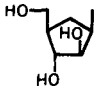
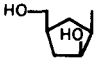
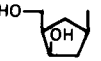
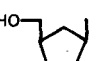
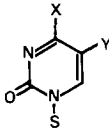




<u>S</u>	<u>Compound No.</u>	<u>X</u>	<u>Ref.</u>
	56a (C-Urd)	H	65
	b	CH ₃	13,66
	c	F	66
	d	Br	66
	e	I	66
	f	NH ₂	66
	g	NHCH ₃	66
	h	CH ₂ OH	66
	57a (C-dUrd)	H	65,66
	b (C-Thy)	CH ₃	13-15,66
	c (5-F-C-dUrd)	F	66,67
	d	Br	66
	e	I	66
	f	NH ₂	66
	g	NHCH ₃	66
	h	NHC ₂ H ₅	66
	i	N(CH ₃) ₂	66
	58a	H	65,66
	b	CH ₃	13,15,66
	c	F	66,67
	d	Br	66
	e	I	66
	f	NH ₂	66
	g	NHCH ₃	66
	59	H	68
	60	CH ₃	15
	61	CH ₃	15
	62	CH ₃	15

Table II.
(Continued)

					
	63a (C-Cyt)	NH ₂	H	69	
	b	N(CH ₃) ₂	H	70	
	c	NH ₂	CH ₃	68	
	d	NH ₂	NHCH ₃	68	
	e	NH ₂	N(CH ₃) ₂	68	
	f	OMe	H	68	
	64a	NH ₂	H	70	
	b	N(CH ₃) ₂	H	70	
	65a	NH ₂	H	70	
	b	N(CH ₃) ₂	H	70	
	66 (C-Ara-C)	NH ₂	H	70,71	
	67	NH ₂	H	70	

parts. Other variations include substitutions at positions 6, 2, and 8 which are indicated as X, Y, and Z (Table 1).

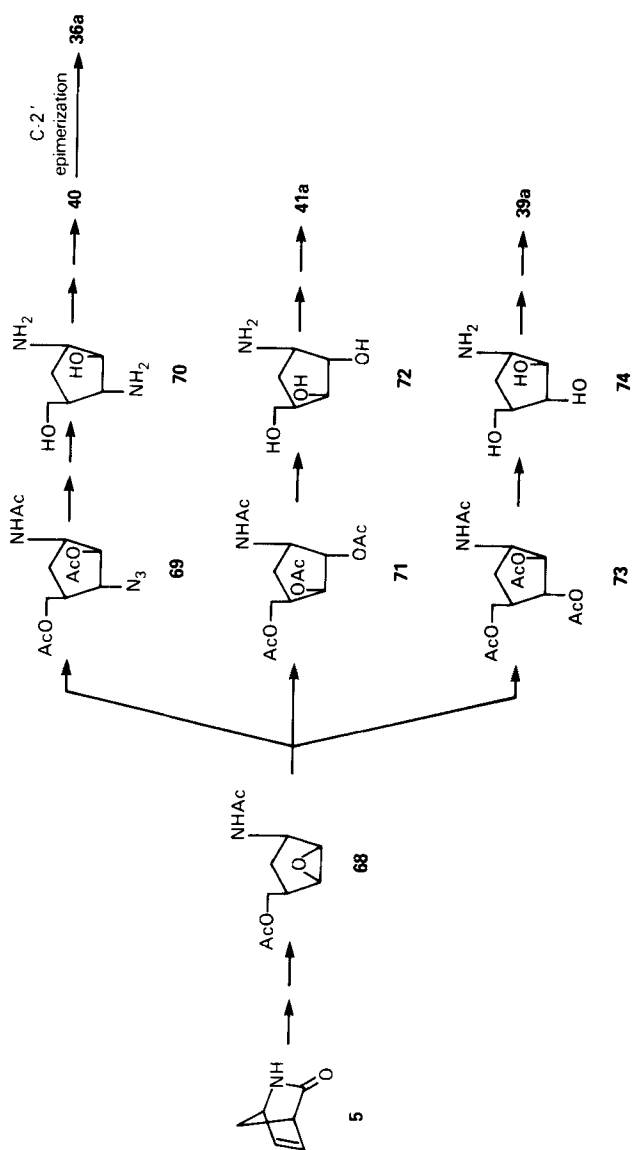
a. Ribose Isosteres

The first compound of this class that was synthesized was the carbocyclic analogue of adenosine (C-Ado, **35a**) which was obtained as a racemate.^{9,10} Shortly after, the pure enantiomer was isolated from the culture filtrate of *Streptomyces citricolor* and confirmed to be (1'R,2'R,3'R,4'R)-9[β-D-2'α,3' α-dihydroxy-4'-β(hydroxymethyl)cyclopentyl]adenine or (-)-aristeromycin.^{5,6} Several other syntheses of C-Ado have been reported including a recent enantioselective preparation.^{16,17,22} As expected, C-Ado displayed a wide range of biological activities.^{54-61a} It was highly cytotoxic to both H.Ep.-2 and L1210 cells in culture, but it demonstrated poor selectivity towards the tumor cells in view of its inactivity in the mouse L1210 model system.¹⁰ At subtoxic concentrations it induced cell proliferation of quiescent normal cells, but in contrast, it inhibited growth in malignant cell lines.^{54a} The primary toxic effects of C-Ado appear to be mainly derived from the corresponding nucleotide (C-AMP) generated in cells containing adenosine kinase (AK⁺).⁵⁴ This metabolite has been synthesized chemically by selective phosphorylation of the 5'-hydroxyl group.⁵⁵ Like adenosine, C-Ado is also deaminated by adenosine deaminase (ADA), but its affinity for the enzyme is a hundredfold lower.⁵⁴ No NAD metabolite derived from C-Ado has been detected and incorporation

into RNA and DNA is negligible. Homopolymerization of the diphosphate (C-ADP) with *E. coli* polynucleotide phosphorylase was also negligible, but in the presence of ADP it was polymerized to polynucleotides containing various ratios of Ado and C-Ado.^{55a} C-AMP could be further metabolized to the triphosphate (C-ATP) and a major metabolic effect appears to be the inhibition of utilization of guanine and hypoxanthine.⁵⁶ Consistent with this observation, C-AMP has been recognized as a powerful inhibitor of GMP kinase ($K_i = 12 \mu\text{M}$) which causes accumulation of GMP. This increased levels of GMP may be responsible for the growth inhibitory properties observed, since GMP is an end-product inhibitor of hypoxanthine(guanine)phosphoribosyl transferase (HGPRT).⁵⁶ A recent reinvestigation of the metabolism of C-Ado in H.Ep.-2 and L1210 cells revealed that in addition to C-ATP, the corresponding carbocyclic guanosine triphosphate (C-GTP) was also formed. This metabolite was found to be the most effective known inhibitor of HGPRT.⁵⁷ As expected, AK deficient cells (AK⁻), which are unable to phosphorylate C-Ado, showed some resistance to the drug after a short exposure.^{54,58,59} However, the ID₅₀ for both AK⁺ and AK⁻ cells after prolonged exposure was very similar (0.3–0.6 μM).⁵⁸ In AK⁺ cells, therefore, rapid toxicity ensues after formation of C-AMP, C-ATP and C-GTP. In AK⁻ cells, on the contrary, there is a slower process mediated by C-Ado itself that does not require metabolic activation. Such delayed cytotoxicity is related to the inhibitory effect of C-Ado on S-adenosylhomocysteine hydrolase (AdoHcy-ase) which will be discussed in Section VIII. C-Ado was also found to be a potent inhibitor of the growth of plant infecting bacteria and a moderate inhibitor of the growth of the protozoon *Tetrahymena pyriformis*.^{60,60a} Lastly, C-Ado showed no significant antiviral activity against HSV, vaccinia, rhino, or influenza viruses.⁶¹

All other 6-substituted C-Ado analogues (**35b–35h**) reported to date were also inactive against L1210 leukemia in mice, despite the fact that some of them (i.e. compounds **35b**, **35c**, and **35f**) were cytotoxic to H.Ep.-2 cells *in vitro*.³⁷ The corresponding carbocyclic inosine (C-Ino, **35d**), although devoid of antitumor activity, showed comparable antiviral activity to that of ara-A against HSV (VR = 2.1).^{61,61b} *In vivo*, C-Ino results from the deamination of C-Ado and is an obligatory intermediate in the metabolic conversion of C-Ado to C-GMP.⁵⁷ Other 6-substituted C-Ado analogues with good antiviral (HSV) activity were **35e** and **35h**.⁶¹ The transformation of the ribose moiety of 6-methylthiopurine ribonucleoside into the cyclopentyl moiety of **35h** resulted in loss of cytotoxicity, but endowed the compound with good antiviral activity which was lacking in the riboside.⁶¹ Simultaneous substitution at C-6 and C-2 engendered the carbocyclic guanosine analogue (C-Gua, **35i**) and other 2-aminopurine derivatives.^{39,40} C-Gua was found neither cytotoxic to H.Ep.-2 *in vitro*, nor active against murine L1210 *in vivo*.^{39,40} The rest of the 2,6-disubstituted analogues (i.e. **35i–35n**) were likewise inactive against L1210 in mice.³⁹ On the other hand, the carbocyclic analogue of 2,6-diaminopurine ribofuranoside (**35i**) demonstrated superior activity to that of ara-A against HSV-1 and vaccinia virus (VR = 4.6) and some of the other derivatives also displayed good antiviral activity (**35j** and **35k**).³⁹

C-Ado analogues substituted at C-8 by either Br (**35o**) or OH (**35p**) have been reported but their biological activity is not known.³⁸



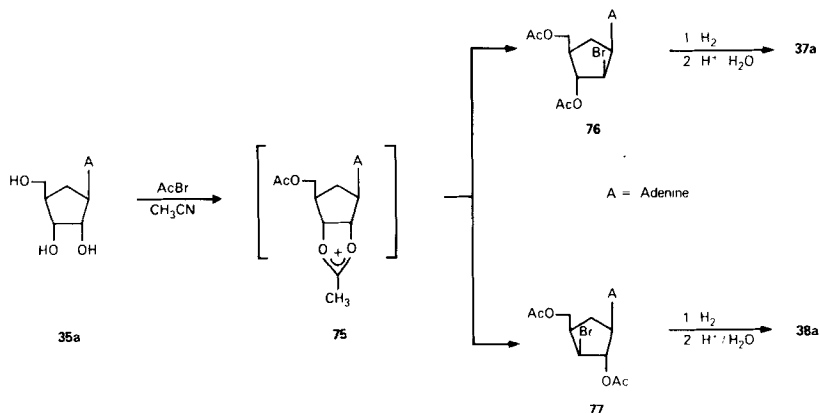
Scheme VIII

b. Other Sugar Isosteres

Replacement of the 3'-OH group by an amino group in the structure of C-Ado generated the carbocyclic 3'-amino-3'-deoxy adenosine (**36a**) which showed good antiviral activity against HSV-1 (VR = 2.1) and vaccinia (VR = 2.2).^{27,29} Such a replacement was performed by Daluge and Vince starting with the versatile 2-azabicyclo[2.2.1]hept-5-ene-3-one (**5**) already discussed in Section II-B. As shown in Scheme VIII, hydrolysis of this compound was followed by the regioselective formation of epoxide **68**. This critical epoxide also served as the starting material for other carbocyclic sugar isosteres.²⁷⁻²⁹ Opening of the epoxide ring with sodium azide occurred predominantly at the position farthest away from the acetamido group to give **69**, whereas hydrolysis and reacylation gave a mixture of **71** and **73**. Reduction of the azide and anchimerically assisted hydrolysis of the acetamide group by the *cis* hydroxyl substituent gave the diamine **70**. This compound was converted to **40** and finally to **36a** after epimerization at C-2'.^{27,29}

Shealy and O'Dell converted *exo*-bicyclo[2.2.1]hept-5-en-2-ol to racemic mixtures of 4-amino-3-hydroxy and 4-amino-2-hydroxycyclopentanemethanol in the manner described for the carbocyclic ribofuranosylamine **6** (Scheme I).^{41,62} These amines served respectively as precursors for the carbocyclic analogues of 2'-deoxy and 3'-deoxyadenosine (**37a** and **38a**). Alternatively, these compounds were accessed directly from (-)-aristeromycin after treatment with acetyl bromide.³⁸ As shown in Scheme IX, bromide attack on the generated acetoxonium ion **75** produced 2' and 3'-bromonucleosides **76** and **77** with inverted configuration. Hydrogenolysis of these halogenonucleosides accomplished the necessary dehalogenation to afford 2' and 3'-deoxyaristeromycin (**37a** and **38a**).³⁸ No report on the biological activity of either 2'-deoxy or 3'-deoxy-C-Ado has appeared.

Recently, several 2'-deoxy carbocyclic nucleosides of 2-amino-6-substituted purines have been reported and the chemical methods of syntheses parallel those of the analogous carbocyclic ribofuranoside isosteres.⁴² These analogues (**37c-f**) are active against HSV-1 and even more potent against HSV-2 than the carbocyclic 5-substituted-2'-deoxyuridines (Section IV-A). Moreover, the deaminated products formed after reaction with adenosine deaminase (ADA)



Scheme IX

retained activity as opposed to the case of the ribofuranoside isostere **35i** which after deamination produced the inactive guanosine derivative **35l**. The compounds, however, were devoid of *in vivo* antitumor activity against murine L1210 leukemia.⁴²

Two very useful modifications of the carbocyclic moiety have been the inversion of configuration of the 2'-hydroxyl and 3'-hydroxyl groups to give, respectively, the deaminase resistant arabinofuranosyl and xylofuranosyl carbocyclic isosteres **39a** and **41a**.^{28,45} As reviewed in Scheme VIII, these compounds originated from the corresponding carbocyclic amines **72** and **74** obtained from hydrolysis of epoxide **68**. C-ara-A (**39a**), in particular, has demonstrated antiviral activity against HSV and vaccinia virus (VR's = 1.5–3.5)²⁸ and more recently its prodrug 5'-methoxyacetate exhibited significant efficacy in the topical treatment of genital infections by HSV-2.⁶³ In addition, it demonstrated activity against the acyclovir-resistant variants deficient in DNA polymerase or thymidine kinase.⁶³ A recent patent describing compounds **39a–e** has appeared but only the activity of C-ara-A (cyclaradine, **39a**) was reported.⁴³ Both isosteres (C-ara-A and C-xylo-A) were cytotoxic to P388 mouse lymphoid leukemia cells,^{43,45} but no *in vivo* activity was evidenced.⁴⁵

Another carbocyclic arabinoside analogue with good antiviral activity was the 2,6-diaminopurine analogue **39f** (VR = 1.5).⁴⁴ The carbocyclic riboside isostere **35i**, however, appears to be superior.³⁹ Carbocyclic C-ara-Gua (**39g**,) on the other hand, demonstrated good cytotoxicity against P388 cells *in vitro* at a concentration five times lower than that of **39f**, but lacked antiviral activity.⁴⁴

A final modification of 2' and 3'-hydroxyl groups in C-Ado included the simultaneous inversion of these groups as in the lyxo compound **42a**. No biological data has been reported for this compound.³⁰ The deletion of both 2' and 3'-hydroxyl groups, resulting in compounds **43a–f**, produced only weak inhibitors of ADA.¹²

B. 8-Azapurine Carbocyclic Nucleosides

This group includes carbocyclic analogues of 8-azapurine ribonucleosides with variations on the carbocyclic moiety typical of the nucleoside counterparts. Substituents on the 8-azapurine ring are at positions 2 and 6 (Table I).

a. Ribose Isosteres

The simplest member reported in this series is compound **44a** (8-aza-C-Ado)^{46,47} which represents one of the few carbocyclic purine derivatives active *in vivo* against P388 (max ILS 68% at 100 mg/Kg qd 1–9) and L1210 (borderline activity) leukemias. The corresponding 8-azahypoxanthine analogue (8-aza-C-Ino, **44b**) was devoid of antitumor activity both *in vitro* and *in vivo*,⁴⁷ but, in agreement with the observed inverse relationship between antitumor and antiviral activities, it displayed good antiviral activity with VR's of 1.5 against HSV and 1.2 against vaccinia virus.⁶¹ Recently, the 8-aza-C-Gua (**44f**) analogue, as well as compounds **44c–e**, were evaluated as antiviral agents.³⁹ Only the 8-aza-C-2,6-diamino analogue **44c** demonstrated activity against HSV-1 (VR = 1.4), but it was less potent than the carbocyclic 2,6-diaminopurine **35i**.³⁹ 8-Aza-C-Gua was devoid of cytotoxicity against H.Ep.-2 cells *in vitro* and was ineffective against L1210 *in vivo*.⁴⁰

b. Other Sugar Isosteres

Typical variations of the sugar moiety represented by the 2'-deoxy and 3'-deoxy analogues of 8-aza-C-Ado (**45a** and **46a**) produced inactive compounds against L1210 leukemia suggesting that these compounds are not being phosphorylated intracellularly.⁴⁷ The corresponding 2-amino-6-substituted-8-aza-C-2'-deoxy analogues (**45c–e**) displayed antiviral activities against both HSV-1 and HSV-2 which were superior to ara-A in VR value (4-5) and potency.⁴² In this case, deamination of **45e** by ADA, generated an even more potent antiviral compound (**45d**). The carbocyclic arabinose isostere of 8-azaguanosine (8-aza-C-ara-Gua, **47a**) was cytotoxic to P388 cells *in vitro* as was C-ara-Gua and it was similarly devoid of antiviral activity.⁴⁴ Conversely, the 2,6-diamino-8-aza-ara carbocyclic analogue **47b**, displayed very low cytotoxicity to P388 cells and significant activity against HSV-1 with a VR of 1.3.⁴⁴ Change of the carbocyclic moiety to the xylo configuration as in 8-aza-C-xylo-A,* produced a compound resistant to ADA deamination and with significant *in vivo* antitumor activity against P388 leukemia (ILS 126% at 50 mg/Kg on days 1 and 5).⁴⁵ The activity of this compound varies with the schedule of administration but it is significant enough to deserve further investigation.

Finally, the simultaneous inversion of the 2' and 3'-hydroxyl groups that generated the carbocyclic lyxofuranosyl analogues **48a** and **48b**, plus the complete deletion of these hydroxyl groups altogether (**49a,b**), afforded compounds whose activities were not reported.^{30,47}

C. 3-Deazapurine Carbocyclic Nucleosides

The parent compound in this series is 3-deaza-C-Ado (**50a**) which was found to be a good antiviral agent against HSV-1, vaccinia virus, and HL-23 type virus.⁴⁸ The antiviral activity and potency against vaccinia (VR = 2.8, MIC₅₀ = 1.9 ug/ml) compared favorably with respect to that of ara-A (VR = 1.2, MIC₅₀ = 19.2 ug/ml) but it was less potent than ara-A against HSV-1. In contrast to C-Ado, 3-deaza-C-Ado is neither deaminated by ADA nor phosphorylated by AK in L1210 cells.⁴⁸ The relationship between its antiviral activity and inhibition of AdoHcy-ase will be discussed separately (Section VIII). Precursors and derivatives of 3-deaza-C-Ado are listed in Table I (**50b–d**).

D. 7-Deazapurine Carbocyclic Nucleosides*a. Ribose Isosteres*

The prototype of this series is 7-deaza-C-Ado (**51a**, Table I) which was recently reported together with its 6-chloro substituted precursor (**51b**).⁴⁹ As expected, the compound was resistant to ADA in the same manner as the ribofuranoside isostere tubercidin (7-deazaadenosine) and it required phosphorylation to exert its cytotoxic effect. In *in vivo* tests it was toxic to mice but ineffective in prolonging the life of P388 infected mice.⁴⁹ Its antiviral activity was also marginal. The 8-methyl analogue **51c** has also been reported recently and likewise it was found neither cytotoxic to L1210 cells nor active against HSV-1.⁵⁰

*This compound was inadvertently omitted from Table I. Its correct placement in this Table would be following compound 47.

b. Other Sugar Isosteres

The carbocyclic ara and lyxofuranosyl analogues **52a-d** and **53a,b** have been prepared and found inactive as antitumor or antiviral agents.^{50,51} Removal of both 2' and 3'-hydroxyls gave **54** which was equally inactive against L1210 cells *in vitro*.⁵²

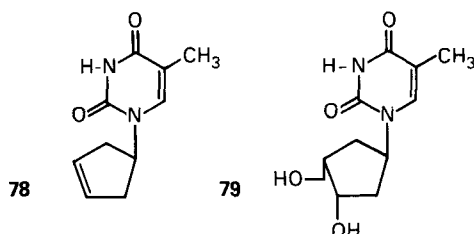
E. 1-Deazapurine Carbocyclic Nucleosides

Only one compound in this group has been reported thus far and it is the corresponding 1-deazaaristeromycin (**55**, Table I). It was prepared in an analogous manner as C-Ado starting with 4-amino-2-chloro-3-nitropyridine and C-rib-NH₂ (See Scheme V).⁵³ The compound was found to be a weak competitive inhibitor of ADA and a potent irreversible inhibitor of AdoHcy-ase with poor affinity for the receptor.⁵³ No other biological data is yet available.

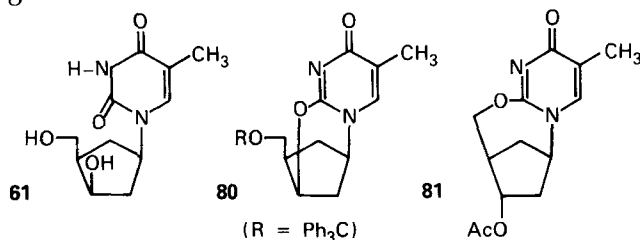
IV. PYRIMIDINE CARBOCYCLIC NUCLEOSIDES

A. Uracil Series

This group includes all the cyclopentyl derivatives of substituted and unsubstituted 2,4-(1H,3H)pyrimidinediones that mimic the different pentofuranosyl nucleosides (Table II). Chronologically, the first compound described was the thymidine isostere C-Thy (**57b**) which was synthetically approached by the Prins reaction starting with known 1-(3-cyclopentenyl)thymidine (**78**).^{7,8}



A comparison of the product obtained through this reaction with authentic C-Thy, as prepared later by Shealy and O'Dell,^{13,14} demonstrated that the first sample had structure **79**.¹⁵ The current approach to this class of compounds, including C-Thy, begins with a carbocyclic amine precursor (Scheme VII) as discussed earlier in Section II-C-b. With this approach the generation of anhydronucleosides **80** and **81** from C-Thy further corroborated the correct disposition of the base on the carbocyclic moiety.¹⁵ Additionally, ¹H and ¹³C-NMR studies confirmed the relative disposition of hydroxyl groups on the carbocyclic ring.^{13,15}



The carbocyclic uridine analogue (C-Urd, **56a**) was first reported by Holy,^{20,64} but since the structure of his starting C-rib-NH₂ was later confirmed to be erroneous,²⁵ the isolated compound was rather an isomer of C-Urd of yet unknown structure.² The compound with the correct structure was prepared later by an analogous procedure used for the synthesis of C-Thy that started with authentic C-rib-NH₂ (**6**).^{65,66} The generality of Shealy's method permitted ready access to many carbocyclic sugar isosteres by simply starting with the appropriate aminocyclopentane (**57–62**).^{13,15,65,66} In addition, compounds with inverted configuration at C-2' and C-3' (**59**, **60**, and **61**) were obtained by hydrolysis of the corresponding 2,2'-anhydronucleosides and 2,3'-anhydronucleosides (e.g. **80** → **61**).¹⁵

Substituted carbocyclic uracil nucleosides containing halogens at C-5 (**56c–e**, **57c–e** and **58c–e**) were obtained by direct halogenation of the parent unsubstituted compound,^{66,67} including treatment with elemental fluorine to afford the interesting 5-F-C-dUrd (**57c**).⁶⁷ Other C-5 substituents were introduced by displacement of the halogen (Br or I) with ammonia or various amines (**56f,g**; **57f–i**; **58f,g**). The 5-(hydroxymethyl)carbocyclic uracil **56h** was obtained from the acetonide of C-Urd after treatment with paraformaldehyde.⁶⁶

All carbocyclic uracil nucleosides with isosteric ring moieties of ribose, 2'-deoxy, and 3'-deoxyribose (**56a**, **57a**, **58a**) were inactive in culture against KB as well as L1210 cells.⁶⁵ The very attractive 5-F-C-dUrd (**57c**), which is chemically stable and does not release 5-F-Ura, was anticipated to be more specific than 5-F-dUrd. However, it proved to be a rather weak and disappointing analogue of 5-F-dUrd that failed to increase the life span of mice bearing P388 and L1210 leukemias. This inactivity probably results from a low level of phosphorylation to the corresponding nucleotide.⁶⁷ C-Thy (**57b**) was the only member of the pyrimidine series with modest and reproducible activity against *in vivo* L1210 leukemia despite its inactivity against KB and H.Ep.-2 cells in culture.¹³

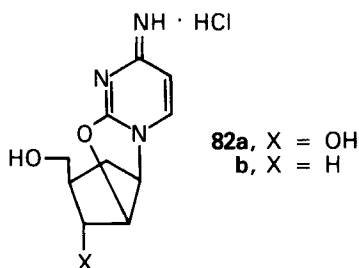
Like their pyrimidine nucleoside counterparts, many carbocyclic isosteres have shown good activity against HSV-1 and HSV-2 viruses (Table III). These carbocyclic analogues appear to be activated by viral-induced thymidine kinase (TK) in view of their inactivity against TK⁻ variants.⁶⁶ The carbocyclic analogue of I-dUrd was the most potent compound of the series.

B. Cytosine Series

The parent carbocyclic cytidine (C-Cyt, carbodine, **63a**) was prepared from the 2',3',5'-tri-O-benzoyl carbocyclic uridine analogue *via* the intermediate 4-chloro derivative after reaction with methanolic ammonia under pressure.⁶⁹ Other N-substituted analogues were obtained similarly by displacement of the chloride with the appropriate amine.⁶⁸ The carbocyclic analogues of 2'-deoxy and 3'-deoxycytidine (**64a** and **65a**) were synthesized by the same procedure from the corresponding uracil derivatives (Table II).⁷⁰ Both carbocyclic isosteres of cytosine arabinoside (C-ara-C, **66**) and the 3'-deoxyarabinosyl analogue **67**, were obtained after hydrolysis of the corresponding 2,2'-anhydrocytidine intermediates (**82a,b**) formed after treatment of carbocyclic cytidines **63a** or **65a** with DMF-thionyl chloride.^{70,71}

Table III
Antiviral Activity of Selected Carbocyclic Pyrimidine Nucleosides

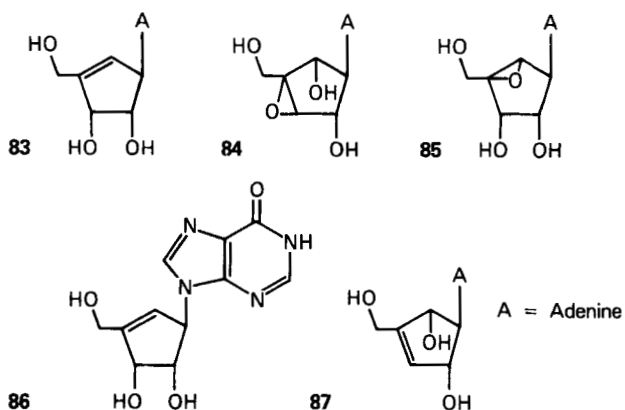
Compound	X	HSV-1		HSV-2	
		VR	MIC ₅₀ (μg/ml)	VR	MIC ₅₀ (μg/ml)
57b	Me	5.4	0.8	3.2	7.0
57d	Br	6.2	0.3	1.5	32
57e	I	7.1	0.3	1.5	20
57g	MeNH	3.9	15		
57h	C ₄ H ₉ NH	2.0	290		
l-d Urd		7.4	0.3	5.0	1.0
ara-A		2.7	9.8	2.3	20.1



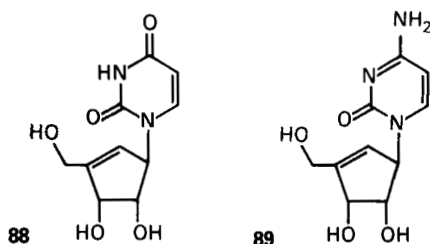
As antitumor agents, the carbocyclic cytidine analogues fared significantly better than their uridine counterparts as both C-Cyt and C-ara-C demonstrated good and reproducible activity against L1210 leukemia in mice. At 200 mg/Kg qd 1-9, C-Cyt gave 82% ILS, whereas C-ara-C at 150 mg/Kg produced 104% ILS.⁶⁹⁻⁷¹ C-Cyt has been more thoroughly studied and it has been shown that L1210 cells phosphorylate it to the mono-, di- and triphosphate level and that it is subject to deamination by cytidine deaminase.^{68,72} Accompanying the formation of C-CTP was a specific decrease in the CTP pool indicating inhibition of the critical step that converts UTP to CTP.⁶⁸ This could be a limiting factor in the synthesis of RNA, but *in vitro* studies with L1210 cells revealed that DNA synthesis was inhibited to a greater extent than RNA synthesis.⁶⁸ Since no significant incorporation into polynucleotides was observed, it is possible that C-CTP may interfere with DNA polymerase.⁶⁸ As an antiviral agent C-Cyt was only marginally active against HSV-1; however, it showed good *in vitro* activity against influenza virus in mammalian cells.⁶⁸ *In vivo* studies against this virus were negative, as well as the *in vitro* combination of C-Cyt with the cytidine deaminase inhibitor tetrahydrouridine.⁶⁸ Both 2'-deoxy and 3'-deoxy isosteres (**64a** and **65a**) were ineffective as either antitumor or antiviral agents.^{68,70}

V. CYCLOPENTENYL NUCLEOSIDES: THE NEPLANOCINS

The neplanocin antibiotics comprise a group of compounds with variations in their carbocyclic moiety that depart significantly from the conventional carbocyclic nucleosides discussed thus far. Additionally, since they are the subject of much recent attention, they will be reviewed separately. The original members of the neplanocin family, which include neplanocin A-D, were isolated from a soil-derived strain of *ampuriella regularis*.⁷³⁻⁷⁵ The structure of neplanocin A (**83**) has been confirmed by spectral and X-ray analyses^{76,76a,b} whereas the other neplanocins (**84-87**) have been briefly described.^{74,75,76a}



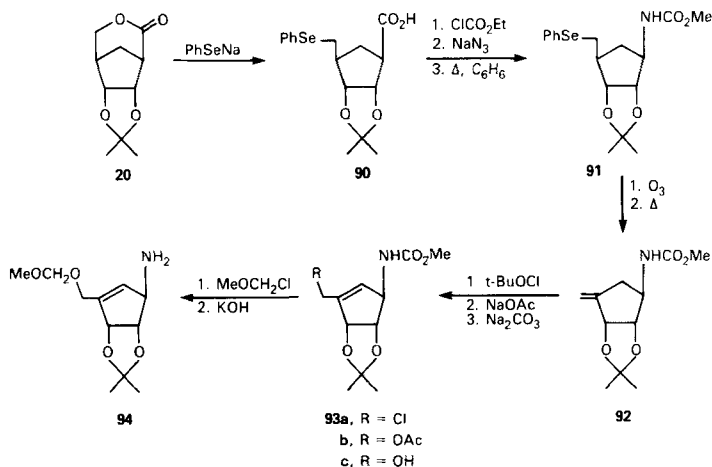
By far the most important member of the neplanocin family is neplanocin A which has demonstrated significant antitumor activity.^{75,76a} Much of the published biological work refers to this compound as simply neplanocin. The neplanocin family has also been extended to other members including the pyrimidine analogues **88** and **89**.^{77,86}



A. Syntheses of Neplanocin

The unprecedented presence of the double bond in the carbocyclic ring moiety of neplanocin expectedly attracted considerable synthetic attention. In 1983 three different total syntheses of neplanocin were reported which opened the route for the syntheses of other congeners.¹⁶⁻¹⁹ The highlights of these syntheses are briefly discussed.

The lactone intermediate **20** reported by Ohno in his synthesis of (-)-aristeromycin (See Scheme IV) was reacted with PhSeNa to afford selenide **90**. After a series of steps, which included a Curtius rearrangement and ad-

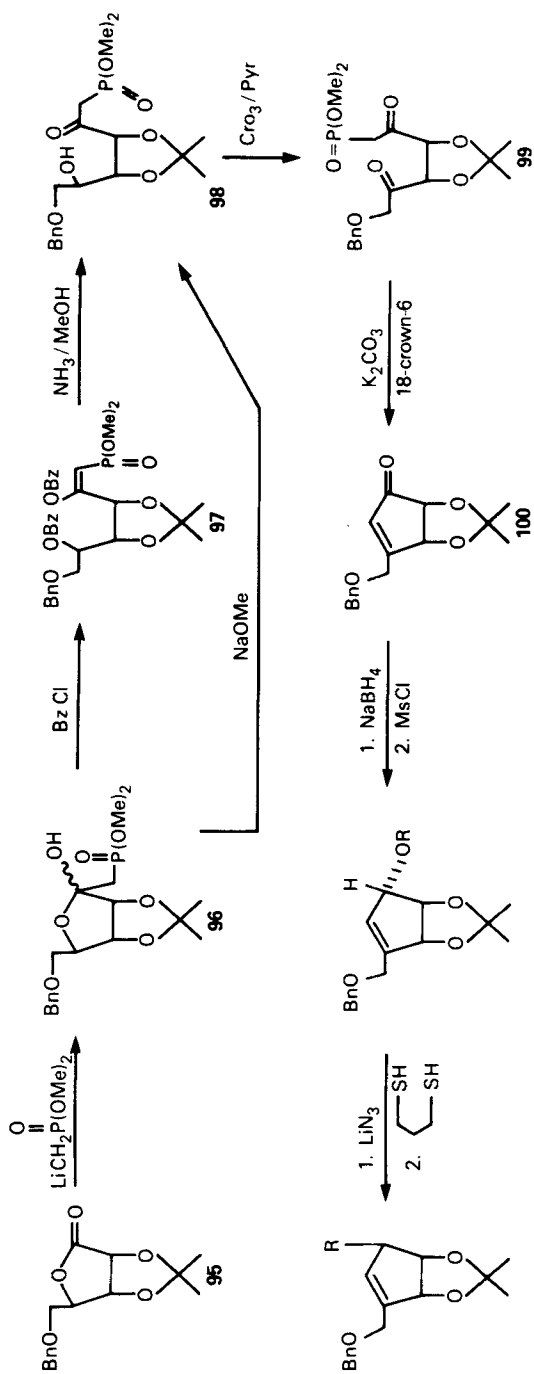


Scheme X
Chemico-enzymatic approach^{16,17}

dition of methanol, the methyl carbamate **91** was obtained. Oxidation of **91** and *syn* elimination of the generated selenoxide afforded compound **92** which contained an exocyclic double bond. Transformation of **92** to the allylic alcohol **93c** was performed in three steps involving an "ene-type" chlorination, displacement with acetate, and hydrolysis. Protection of the allyl hydroxyl in **93c** as the methoxymethyl ether and hydrolysis of the carbamate, afforded the key amine **94**. This intermediate was used to prepare (–)-neplanocin, as well as the guanosine, AICAR, uridine, and cytidine analogues, through the use of conventional chemistry⁷⁷ discussed earlier in this review.

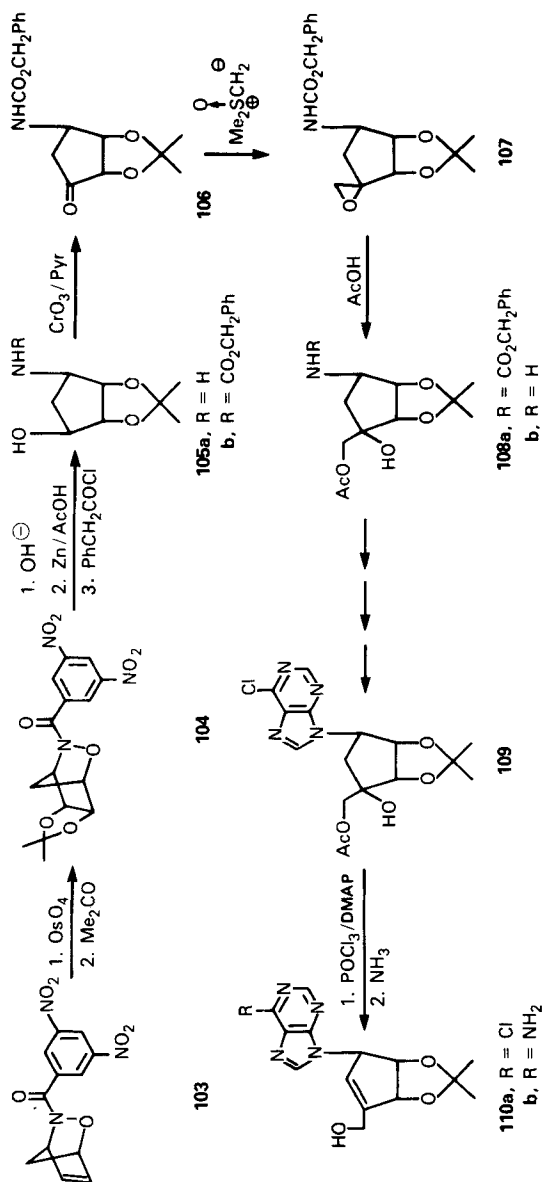
In the chiron approach, use of chiral D-(+)-ribonic acid γ -lactone generated neplanocin in its naturally occurring enantiomeric form. Coupling the protected ribonolactone **95** with lithium dimethyl methylphosphonate, resulted in the formation of hemiketal **96**. Benzoylation of **96** led to the dibenzoate **97** which afforded the acyclic ketophosphonate **98** after deprotection of the benzoyl groups. More conveniently, treatment of **96** with sodium methoxide afforded **98** directly. Oxidation of the alcohol function in **98** produced the 2,5-diketophosphonate **99** which underwent a Wadsworth-Emmons cyclization to the cyclopentenone **100**. In the next step, the concave-convex nature of this bicyclo[3.3.0] system directed the hydride attack from the less congested β -face of the molecule to give the alcohol **101a**. Mesylation of **101a**, followed by S_N2 displacement with N_3^- , afforded azide **102a** which was subsequently reduced to the desired amine **102b** having the β -configuration. This amine was further elaborated into (–)-neplanocin as well as into the corresponding uracil and cytosine carbocyclics.^{18,86}

In the nitron approach the masked amino group of the carbocyclic amine was incorporated in the starting material. (\pm)-3-(3,5-Dinitrobenzoyl)-2-oxa-3-azabicyclo[2.2.1]-hept-5-ene (**103**), which was easily available from a Diels-Alder condensation of cyclopentadiene and the acylnitroso derivative of 3,5-dinitrobenzoic acid, was subjected to a specific *exo-cis* dihydroxylation to the corresponding diol. Acetonide protection of this diol, followed by alkaline



Bn = Ph CH₂
 Bz = Ph CO

Scheme XI
 Chiron approach^{18,78}



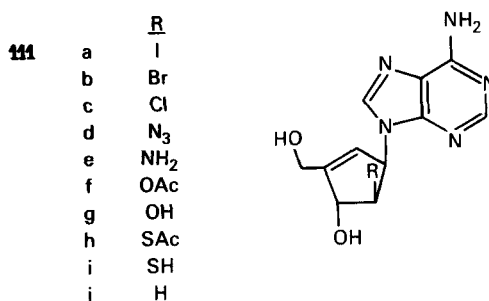
Scheme XII
Nitronone approach¹⁹

hydrolysis of the dinitrobenzamide and reductive cleavage of the N-O bond, afforded amine **105a**. Protection of this amine and oxidation of the hydroxyl function resulted in the formation of cyclopentanone **106**. Reaction of this cyclopentanone with the sulfonyl chloride, dimethylsulfoxonium methylide, resulted in the regioselective formation of epoxide **107**. Opening of the epoxide ring in glacial acetic acid, followed by deblocking of the amino protecting group, produced amine **108b** upon which the purine ring was constructed. Dehydration of the tertiary alcohol in **109** afforded the desired carbocyclic skeleton and ammonolysis performed on the resulting product yielded the protected racemic neplanocin **110b**.

B. Neplanocin Analogues

Several purines and pyrimidines bearing the cyclopentenyl moiety have been prepared and many more are to be expected in the near future. Among the purine analogues that deserve special mention are a series of 2'-modified carbocyclic isosteres represented by structure **111**. Simultaneous protection of the 3' and 5'-hydroxyls of neplanocin with Markiewicz's reagent (1,3-dichloro-1,1,3,3-tetraisopropylidisiloxane) permitted the derivatization of the 2'-hydroxyl into a leaving group that yielded compounds **111a-j** after reaction with various nucleophiles.^{79,80,80a} 2'-(R)-Mercapto-2'-deoxyneplanocin (**111i**) was characterized by X-ray analysis and the similarity of its CD spectrum with those from other members of the series confirmed the structure of these analogues.⁸¹ The 2'-deoxy isostere was produced from the halogen-containing compounds (**111a-c**), or from the corresponding 2'-O-thiocarbonylimidazolyl derivative, after reduction with tri-n-butyltin hydride.⁷⁹ 3'-Deoxy neplanocin, the analogue of cordycepin, was obtained by a similar procedure.^{80a}

Other modified neplanocins including the 5'-amino and 5'-azido-5'-deoxyneplanocins have been reported in several patents.⁸²⁻⁸⁴



C. Biological Activity

a. Purine Analogues

Neplanocin A was reported to have a striking effect in increasing the life span of mice bearing L1210 leukemia (ILS 120% at 5 mg/Kg, qd 1-5) with a rather broad margin of safety.^{75,76a} It was nevertheless deaminated to the inactive inosine isostere (neplanocin D, **86**), but with a V_{max} 60-fold lower than adenosine.⁷⁵ At the National Cancer Institute neplanocin demonstrated somewhat less pronounced activity against both L1210 (ILS 62% at 4 mg/Kg)

and P388 (ILS 53% at 4 mg/Kg) leukemias. In addition, it was inactive against a host of other tumors including xenografts.⁸⁵

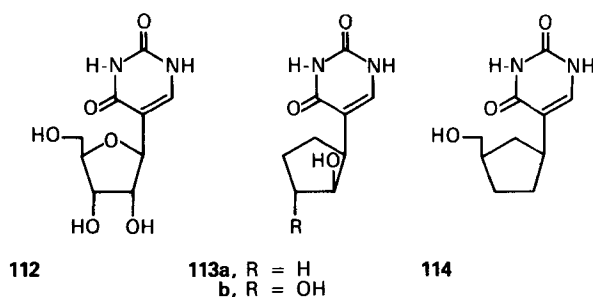
b. Pyrimidine Analogues

The only compounds reported thus far in this series are the uridine and cytidine isosteres **88** and **89**. Their *in vitro* study with KB and L1210 cells revealed that only the cytidine analogue was cytotoxic.^{77,86} The IC_{50} for this compound against L1210 was 30 nM, while the uridine analogue inhibited cell growth only 12% and 36% at 0.5 mM and 1 mM, respectively.⁸⁶ Both compounds, however, behaved as competitive inhibitors of uridine kinase with K_i values of 60 μ M (cytidine analogue **89**) and 230 μ M (uridine analogue **88**) and likewise capable of selectively inhibiting uridine and cytidine salvage in intact cells.⁸⁶ When compared with the saturated carbocyclic isosteres **56a** ($K_i = 930 \mu$ M) and **63a** ($K_i = 250 \mu$ M) the level of kinase inhibition was superior for the unsaturated analogues by a factor of 4.⁸⁷ The cytotoxicity associated with the cytidine isostere appears to be related to potent inhibition of CTP synthesis which occurred at doses tenfold lower than with carbodine (C-Cyt, **63a**) in HT-29 human colon carcinoma cells.^{88,89} In addition, drug exposure for 24 h with this compound completely inhibited DNA synthesis in these cells at concentrations of the drug ca. 0.25 μ M.⁸⁸

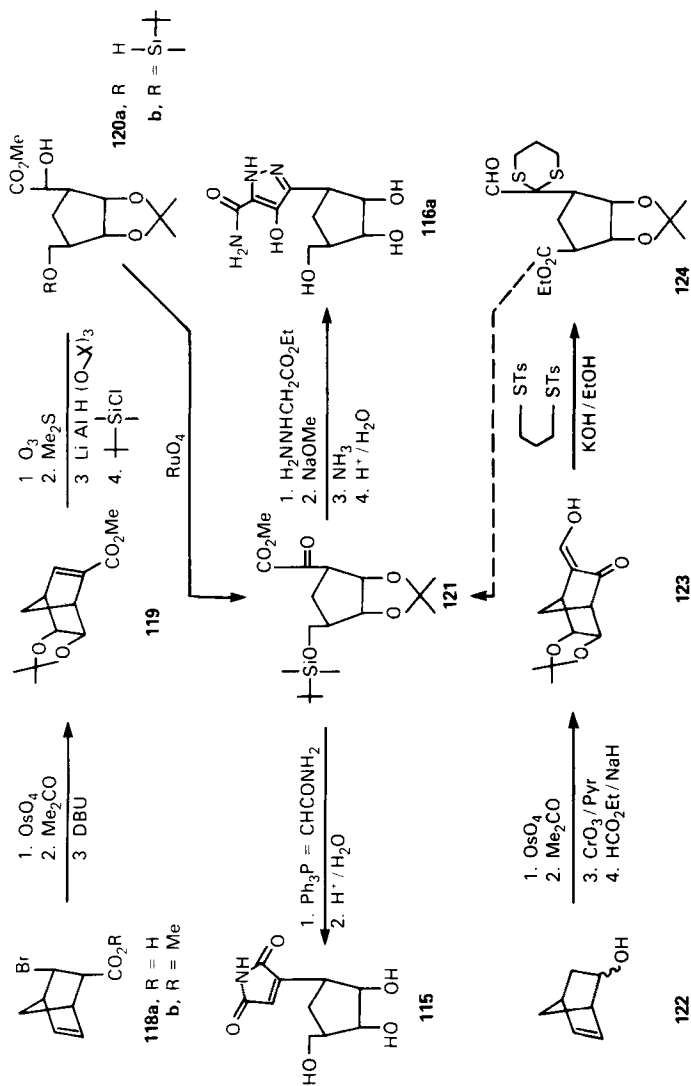
Some recent unpublished observations appear to indicate that this compound is also a powerful differentiator of HL-60 cells, producing even a greater effect than retinoic acid.⁹⁰

VI. CARBOCYCLIC ANALOGUES OF C-NUCLEOSIDES

Since the isolation of pseudouridine (**112**) a new class of nucleosides was discovered in which the characteristic C-N linkage between sugar and base was replaced by a C-C bond. These carbon nucleosides, or C-nucleosides, such as formycin, formycin B, showdomycin, pyrazofurin, and oxazinomycin, possess antibiotic properties and many exhibited antiviral or anticancer activities.⁹¹ The chemistry and biological properties of C-nucleosides have been described in several reviews.⁹²⁻⁹⁵

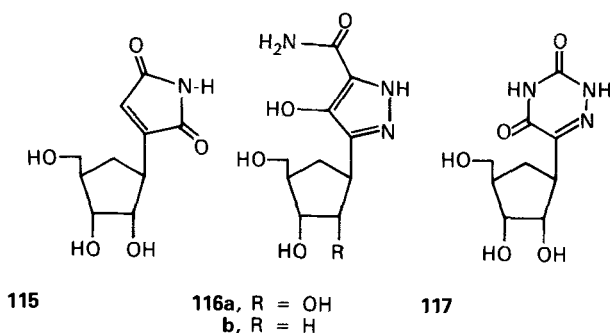


A logical extension in the quest for new synthetic bioactive molecules was to incorporate the carbocyclic moiety into this class of compounds. As early as 1966, some carbocyclic 5-monohydroxycyclopentylpyrimidines (**113a**) and 5-dihydroxycyclopentylpyrimidines (**113b**), which bear a structural similarity to pseudouridine, were prepared.^{96,97} These compounds together with the



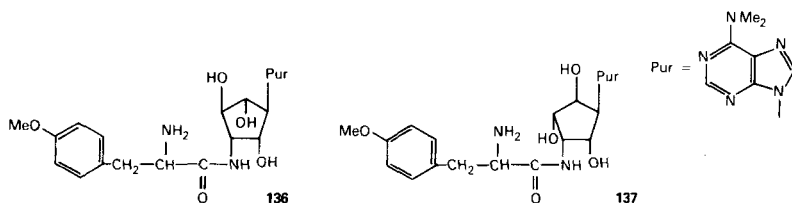
Scheme XIII

carbocyclic analogue of 2',3'-dideoxypseudouridine **114** were used as probes to study the intramolecular H-bonding capacity of pseudouridine.⁹⁸ A more systematic approach to carbocyclic C-nucleosides was begun by Just and co-workers who prepared carbocyclic isosteres of DL-showdomycin (**115**),⁹⁹ DL-pyrazofurin (**116a**),^{99,100} 2'-deoxy-DL-pyrazofurin (**116b**),¹⁰¹ DL-6-azapseudouridine (**117**),¹⁰⁰ 2-thio-6-azapseudouridine¹⁰⁰ and several others.^{102,103} In a manner reminiscent of Shealy's approach, the Diels-Alder adduct **118a**, obtained from cyclopentadiene and bromoacrylic acid, was used as the starting point (Scheme XIII).^{104,105} Following *cis* hydroxylation of the corresponding ester **118b** with OsO₄, the resulting *exo* diol was protected as the isopropylidene derivative, and after base catalyzed elimination of HBr, the olefin ester **119** was obtained. Ozonolysis, followed by reduction with dimethyl sulfide gave the corresponding ketoaldehyde which was further reduced to the diol **120a**. Selective protection of the primary alcohol to give **120b** and oxidation of the secondary alcohol, afforded the key α -ketoester **121**. This α -ketoester was used in the synthesis of the carbocyclic isosteres of showdomycin (**115**) and pyrazofurin (**116a**).^{99,101} Independently, Saksena arrived at the critical precursor to intermediate **121** by a different route as shown in Scheme XIII.¹⁰⁶ Continued interest in the synthesis of these structurally interesting compounds was reduced by the lack of biological activity displayed by the first prototypes in preliminary studies.⁹⁹

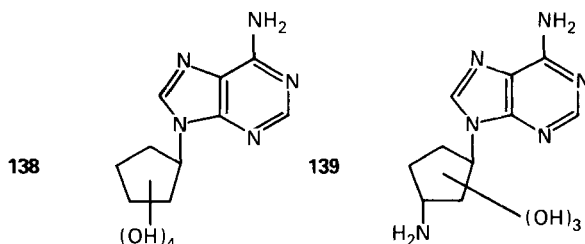


VII. MISCELLANEOUS

The term carbocyclic nucleoside can also be extended to include a series of compounds with altered carbocyclic moieties that significantly separate them from the rest of the other carbocyclic ribose isosteres in that they lack the hydroxymethyl group that permits formation of the corresponding nucleotide analogue. For this reason they will be reviewed separately, and not as extensively, except in some important cases where the lack of such functionality proved to be biologically advantageous. A case in point was the puromycin analogue **125a** which displayed antimicrobial activity virtually identical to that of puromycin but with the advantage that after metabolic removal of the amino acid moiety, the released aminonucleoside was resistant to kinase activity and unable to generate the nephrotoxic aminonucleotide which was otherwise generated from puromycin.¹⁰⁷ Two syntheses have been reported for this compound (Scheme XIV) both of which produced the racemate.^{107,108} In one of the approaches, however, compound **135b** was resolved and the



In other reports, analogues of 9-(2',3',4',5'-tetrahydroxycyclopentyl) adenines (**138**), derived from five of the ten possible isomers of 5-aminocyclopentanetetrol, were prepared and shown to have some antifungal activity.¹¹³ Similarly, the synthesis of analogous 3'-amino-3'-deoxy derivatives (**139**) was accomplished but the compounds were devoid of activity against HeLa cells.¹¹⁴



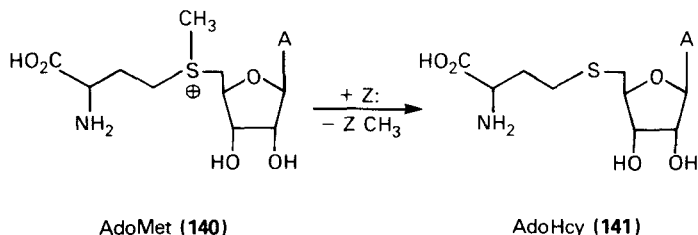
Several more carbocyclic analogues of adenosine and pyrimidine nucleosides in which the ribofuranosyl moiety was replaced by polyhydroxycyclopentyl or polyhydroxycyclohexyl groups have been prepared, but as might be expected, their biological activity was of no significance.^{11,115-117}

VIII. CARBOCYCLIC NUCLEOSIDES AND ENZYMATIC METHYLATION REACTIONS

In view of the very specific inhibition of enzymatic methylation reactions brought about by a group of carbocyclic nucleosides, the aspects concerning this activity will be discussed separately.

A. Background

Very important biological methylation reactions are catalyzed by S-adenosylmethionine (AdoMet)-dependent methyl transferases in which transfer of the methyl group occurs *via* nucleophilic displacement by electron-rich groups (OH, NH, SH or double bonds) present in the methyl acceptor molecule (Figure 2). The methyl acceptor moieties can be small molecules (i.e. triptamine, histamine), medium-size molecules (i.e. phospholipids), or macromolecules (i.e. RNA, DNA, and proteins).^{118,118a,120}



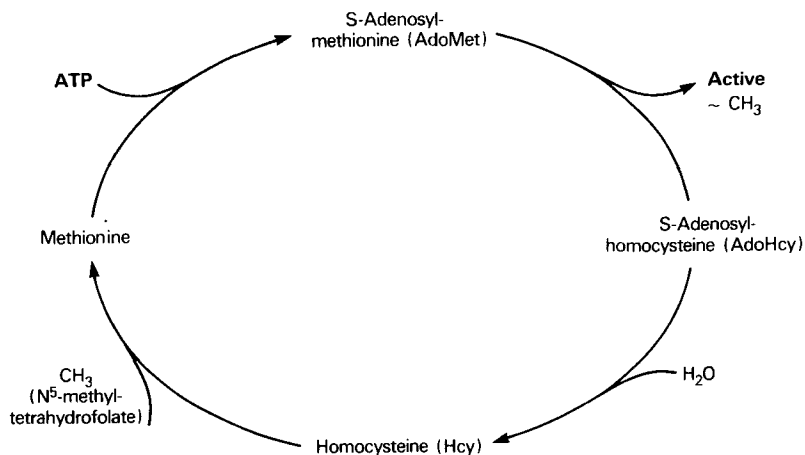


Figure 2. Activated Methyl Cycle.

After methyl transfer, the resulting product from AdoMet (**140**) is adenosylhomocysteine (AdoHcy, **141**) which in turn behaves as a very powerful feedback inhibitor of the methylation reaction.¹¹⁹ Therefore, another enzyme (AdoHcy hydrolase or AdoHcy-ase) efficiently removes this material by cleaving it to adenosine (Ado) and homocysteine (Hcy). Kinetically, however, AdoHcy hydrolysis is a reversible reaction favoring the synthesis of AdoHcy, but its direction is nevertheless forced in the hydrolytic sense due to the efficient removal of Ado and Hcy by further metabolism (i.e. deamination or phosphorylation of Ado and remethylation of Hcy back to methionine).¹²⁰ Inhibition of AdoHcy-ase will therefore result in increased levels of AdoHcy and consequent inhibition of methylation.¹²¹ Furthermore, since cells cannot eliminate AdoHcy across the membrane, the effects of inhibition of methylation are expected to be relatively long lasting.¹²² Inhibition of methyltransferases *via* alteration of the AdoHcy metabolism has attracted considerable attention as a target for drug design particularly in relation to the inhibition of a critical methylation reaction necessary for the proper processing of viral messenger RNA.^{123–125} These mRNA molecules must be capped and methylated at their 5'-terminus in order to initiate translation into viral proteins.¹²⁶ Not surprisingly, therefore, many potent inhibitors of AdoHcy-ase have shown good antiviral activity against viruses requiring a methylated 5'-cap mRNA.^{48,121,125,127} Also, the biochemical connection between methylation patterns in macromolecules, particularly in RNA and DNA, in tumors as well as in cells transformed by oncogenic viruses, has heightened the importance of AdoHcy-ase as a target for chemotherapeutic agents.^{128,129,129a} A matter of concern, however, is the possible lack of specificity resulting from an indiscriminate inhibition of other methylases due to the increased levels of AdoHcy.^{118,130}

B. Mechanism of Adenosylhomocysteine Hydrolase (AdoHcy-ase)

The mechanism of AdoHcy-ase was elegantly elucidated by Abeles and Palmer who demonstrated that the reaction proceeds with the temporary oxidation of the C-3' hydroxyl of the substrate in either direction (AdoHcy or Ado) through the action of enzyme-bound NAD (Figure 3).^{131,132} This

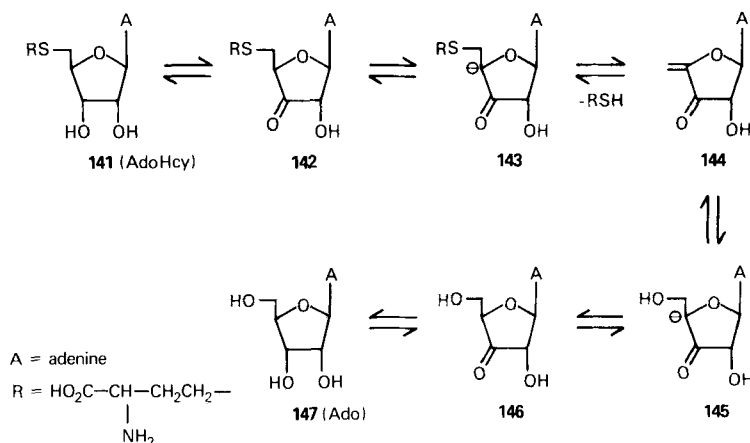


Figure 3. Mechanism of AdoHcy-ase.

oxidation facilitates the elimination of Hcy from AdoHcy, or the hydroxyl group from Ado, after a base-catalyzed removal of the C-4' proton to give a 3'-keto-4',5'-dehydroadenosine intermediate **144**. A Michael-type addition on this intermediate ensues by either water or Hcy, followed by the subsequent reduction back to the ribose moiety mediated by NADH. By this strategy the enzyme converts a difficult displacement reaction into a more facile elimination-and-addition reaction. The K_m 's for AdoHcy and Ado are, respectively, 10.5 μM and 45 μM for the beef liver enzyme.¹³²

C. AdoHcy-ase Inhibitors

An important number of purine nucleosides, analogous to adenosine, interfere with AdoHcy-ase resulting in various degrees of inhibition of AdoMet-mediated methylation reactions.¹³³⁻¹³⁶ This phenomenon is thought to be the direct consequence of increased levels of AdoHcy which change the critical AdoMet/AdoHcy ratio.¹¹⁸ Many of these nucleosides also serve as substrates for AdoHcy-ase, thus, generating novel 5-nucleosidylhomocysteine analogues (NucHcy's) from the purine nucleosides and Hcy.¹³³⁻¹³⁵ However, it appears for the most part that the elevation of AdoHcy levels and not the novel metabolites are chiefly responsible for the inhibition of methylations. The inhibition of AdoHcy-ase occurs by a nucleoside dependent mechanism. For example, some of the ribose-modified Ado analogues, such as Ara-A and 2'-dAdo, inhibit the enzyme irreversibly (suicide inhibitors) and do not serve as substrates for the enzyme.¹³³⁻¹³⁷ Others function as potent reversible inhibitors, as well as substrates, as is the case of the base-modified analogue 3-deazaadenosine (3-deaza-Ado),¹³⁵ while some purine nucleosides function strictly as substrates without causing any elevation of AdoHcy levels (e.g. nebularine).^{133,134} In a few instances some nucleosides form the NucHcy analogue independently of AdoHcy-ase possibly as a result of transmethylation reactions that use the NucMet analogue as a methyl donor. This NucMet metabolite could be formed from the reaction between the nucleoside triphosphate (substituting for ATP) and methionine.¹³³

Table IV.
Summary of the Biochemical Properties of Purine Nucleoside and Carbocyclic Nucleoside Inhibitors of AdoHcy-ase.

Compound	AdoHcy-ase substrate	AdoHcy-ase (K_i)	Type of Inhibition	NucMet formed	NucHcy formed	Kinase substrate (AK)	ADA substrate
Adenosine	+	$K_m = 45 \mu\text{M}^{132}$		+	+	+	+
2'-Deoxyadenosine	-	$1260 \mu\text{M}^{136}$	irreversible	-	-	+	+
ara-Adenosine	-	$300 \mu\text{M}^{136}$	irreversible	-	-	+	+
3-Deazaadenosine	+	$4 \mu\text{M}^{135}$ $20 \mu\text{M}^{136}$ $1 \mu\text{M}^{135}$	reversible	-	+	-	-
C-Ado (35a)	-	$0.005 \mu\text{M}^{135}$ $0.051 \mu\text{M}^{136}$	irreversible	+	-	+	+
3-Deaza-C-Ado (50a)	-	$3 \mu\text{M}^{48}$ $0.001 \mu\text{M}^{48}$	reversible	-	-	-	-
Neplanocin (83)	-	$0.008 \mu\text{M}^{138}$ $0.021 \mu\text{M}^{135}$	irreversible	+	-	+	+

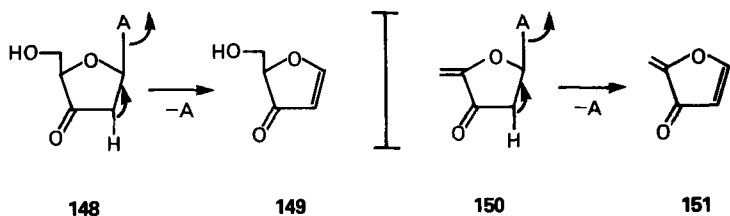
A dramatic change in substrate, binding properties, and mode of inhibition, takes place among some of these nucleosides when the sugar moiety is replaced by a carbocyclic ring. As seen in Table IV the K_i values are often 100 to 1000 times lower. C-Ado is one of the nucleosides with the lowest activity as a substrate for AdoHcy-ase, but in contrast, is one of the most potent inhibitors of the enzyme.¹³⁵ Another carbocyclic nucleoside analogue is 3-deaza-C-Ado (**50a**), which like its riboside isostere 3-deazaadenosine, is resistant to both adenosine deaminase (ADA) and adenosine kinase (AK) besides serving as a poor substrate for AdoHcy-ase.⁴⁸ Although its K_i is not as impressive, the compound appears to be more specific and superior to C-Ado in intact cells.^{134a} It does not inactivate the enzyme and conversion to the NucHcy metabolite does not readily take place.⁴⁸ The antiviral activity of 3-deaza-C-Ado which probably results from the increased levels of AdoHcy and consequent inhibition of methylation of the 5'-cap of viruses, was discussed previously (Section III-C).^{48,127} The chemical syntheses of NucHcy metabolites of C-Ado and 3-deaza-C-Ado have been reported.^{48,135a,b} Of particular interest was the finding that the aristeromycinyl homocysteine analogue of AdoHcy was a potent inhibitor of several methyltransferases displaying different degrees of selectivity towards each enzyme.^{135b} The synthesis of the NucHcy analogue of **43d** containing a plain cyclopentyl ring was also reported.^{135c} The additional presence of the double bond in the carbocyclic structure represented by neplanocin, produced even further changes in activity. The inhibitory activity against AdoHcy-ase was comparable to that of C-Ado ($K_i = 0.0089 \mu\text{M}$) and appeared to be consistent with irreversible inhibition (tight binding) of the enzyme.^{136,138} Like C-Ado, neplanocin is deaminated and phosphorylated to the triphosphate level; however, some important differences exist between these two compounds. First and foremost, C-Ado, although cytotoxic to H.Ep.-2 and L1210 cells *in vitro*, is completely devoid of *in vivo* antitumor activity.¹⁰ Neplanocin, on the contrary, possessed significant antitumor activity *in vivo* against murine leukemias.⁷⁵ Also, as expected for an inhibitor of AdoHcy-ase, neplanocin displayed potent antiviral activity against vaccinia virus in mouse L-929 cells with little or no toxicity to

the host cell.¹³⁸ Consistent with its potent inhibitory activity against AdoHcyase, neplanocin produced a dramatic inhibition of the hydrolase in infected L-cell cultures resulting in a 10-fold increase in the intracellular AdoHcy/AdoMet ratio after a 24 h exposure of 1 μ M of drug.¹³⁸ Neplanocin is phosphorylated but does not appear to be incorporated into RNA or DNA as was the case with C-Ado.¹³⁹ It was, however, highly toxic to Chinese hamster ovary cells (MIC = 0.1 μ M) and cytotoxicity to cells lacking the kinase remained almost unchanged (MIC = 0.5 μ M).¹⁴⁰ Despite the fact that neplanocin is deaminated by ADA to its inactive inosine analogue, addition of ADA inhibitors did not enhance its toxicity.¹³⁸⁻¹⁴⁰ On the other hand, neplanocin was not highly cytotoxic to HT-29 cells when compared to other adenosine analogues and its principal effect was inhibition of RNA methylation.¹³⁹ As with C-Ado, it was not a good substrate for AdoHcyase and consequently the NucHcy analogue was not apparently formed.¹³⁹ This NucHcy metabolite was nevertheless detected as a consequence of a transmethylation reaction that used the NucMet analogue as a methyl donor species.¹³⁹⁻¹⁴¹ In fact, this NucMet metabolite appeared to be the principal metabolite in both HT-29 cells as well as in mouse L-929 cells.^{139,141} This would indicate that the NucMet anabolite can interfere at yet another level of the methylation reaction by directly competing with AdoMet, or blocking it after irreversibly binding to the enzyme.

Inhibition of AdoMet decarboxylase, such as demonstrated for 3-deaza-Ado, 3-deaza-C-Ado and their NucHcy conjugates, could also play a role in the effects of these carbocyclic analogues by affecting polyamine metabolism.^{141a} The activity of neplanocin in this area remains to be studied.

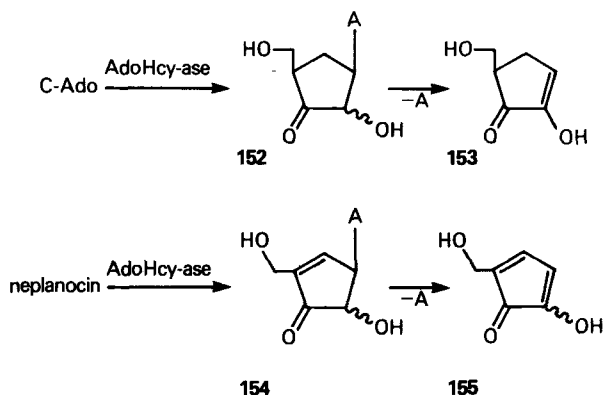
D. Mechanism of Action of AdoHcyase Inhibitors.

In light of the mechanism discovered by Abeles^{131,132} it could be proposed that the inhibitors may (a) compete in a reversible manner with Ado without being essentially transformed by the enzyme (e.g. 3-deaza-C-Ado⁴⁸); (b) compete in a reversible manner with Ado but simultaneously converted by the enzyme to the NucHcy analogue (e.g. 3-deaza-Ado¹³⁴); or (c) compete with Ado and irreversibly inhibit the enzyme by causing it to catalyze its own inactivation (e.g. neplanocin,¹³⁸ 2'-d-Ado¹³⁷). From a mechanistic stand-point, this last group is the most interesting one particularly since most carbocyclic analogues appear to inhibit the enzyme in this suicidal manner. The suicide inactivation of AdoHcyase by 2'-dAdo results from the inherent chemical instability of the enzyme-generated 3'-ketonucleoside **148**.¹³⁷ This intermediate can undergo a favorable *trans*-elimination to give adenine and a sugar residue (**149**). As a result of this elimination, the enzyme is left with its unreacted cofactor (NADH), and hence, it is unable to initiate another cycle



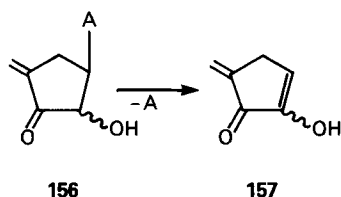
of catalysis due to the fact that its tightly bound NADH cannot be replaced by exogenous NAD.¹³⁷ Despite the apparent reactivity of the resulting sugar residue formed (**149**), there appears to be no covalent modification of the enzyme.¹³⁷ On a closer analysis, however, this α,β -unsaturated system may in fact be chemically deactivated by the furanose oxygen. Nevertheless, according to Abeles, there is also an additional loss of adenine that does not require NAD oxidation and simply results from cleavage of the glycosidic bond. Therefore, an interesting alternative would be the loss of adenine at the next stage of the enzymatic reaction from **150** (formed as depicted in Figure 3 for adenosine) which would lead to the very reactive intermediate **151**. This very intermediate has been suggested as the metabolite responsible for the irreversible inactivation of ribonucleoside diphosphate reductase (CDP and ADP reductase) induced by 2'-F-2'-deoxynucleosides which generate **151** through a different mechanism.^{142,143}

In the case of the carbocyclic analogues, C-Ado and NPC-A, which also inactivate the enzyme in an irreversible manner, there are some mechanistic similarities.^{135,144} For example, addition of either C-Ado or neplanocin leads to an irreversible reduction of enzyme-bound NAD and hence both compounds generate a catalytically incompetent form of the enzyme (NADH form). Also, there appears to be elimination of adenine from **152** or **154** which has to proceed either *via* a *cis*-elimination or a *trans*-elimination (the latter occurring after isomerization of the α -keto hydroxyl group through its enol form).

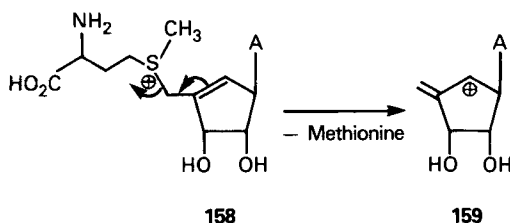


In the case of C-Ado, the generated α,β -unsaturated ketone is also a poor Michael acceptor because the α -OH deactivates the double bond. However, if adenine elimination (*cis* or *trans*) were to take place after the next step of the enzymatic reaction from **156**, it would generate a more reactive species (**157**) capable of binding covalently to the enzyme. The difference in reactivity between **151** and **157** could explain the 10^5 -fold difference between 2'-d-Ado and C-Ado.^{135,137}

In the case of neplanocin, a very reactive α,β -unsaturated system (**154**) could be formed more expediently after oxidation by NAD. Furthermore, if adenine is additionally eliminated, a highly reactive 2,4-cyclopentadien-1-one (**155**) could be generated.



The oxidized form of neplanocin (**154**) could potentially inactivate the enzyme through covalent bond formation, or even react with Hcy to produce a regioisomer of the NucHcy metabolite unable to be reduced back by NADH. On the other hand, if the most important metabolite of neplanocin is the NucMet analogue (**158**),^{139,141} it could also lead to interesting reactions with the methylase due to its reactive double bond. For example, the NucMet metabolite of neplanocin could behave as an inefficient methyl donor which perhaps has the capability of ejecting methionine more readily than the methyl group to give the resonance-stabilized carbocation **159**. The exact mechanisms responsible for the interesting effects observed with neplanocin are still the subject of investigation.



IX. CONCLUSIONS

The isoteric replacement of the furanose oxygen by a methylene group in nucleosides brings about a substantial change in the biological properties of these compounds. Among the carbocyclic isosteres of the naturally occurring nucleosides, only the pyrimidine carbocyclics C-Thy and C-Cyt possessed *in vivo* antitumor properties. In the purine series, C-Ado and C-Gua were both inactive as antitumor agents *in vivo* although C-Ado was highly cytotoxic to L1210 cells *in vitro*. The systematic replacement by the carbocyclic moiety within the ribo, deoxyribo, ara, and xylo series of selected antitumor nucleoside antimetabolites, led in general to a decrease in antitumor activity except in the case of 8-aza-C-xylo-A. On the contrary, good antiviral activity appeared to be the rule rather than the exception among carbocyclic nucleosides. Highlighting the antiviral usefulness of carbocyclic nucleosides are C-Ara-A and 3-deaza-C-Ado among the purines and 5-I-C-dUrd among the pyrimidines.

Carbocyclic analogues whose structure departed significantly from that of a sugar moiety (i.e., plain cyclopentyl groups lacking the 4'-hydroxymethyl substituent) were generally ineffective with the only exception of the puromycin analogue **125a**. Similarly, carbocyclic isosteres of active antitumor carbon nucleosides, such as showdomycin and pyrazofurin, proved to be inactive. Perhaps when the imagination of the medicinal chemist was drawing near to the end in terms of variations and permutation of substituents to be

performed on the carbocyclic moiety, nature sparked the series into a new domain with the structure of neplanocin. In comparison with the twenty year-old field of conventional carbocyclic nucleosides, the cyclopentenyl, or neplanocin series, is in its infancy. Irrespective of whether or not any of the neplanocin analogues reaches clinical status, the initial results with the few cyclopentenyl purines and pyrimidines examined thus far promises to provide the experimentalist with new powerful and sophisticated tools to probe into important biological mechanisms such as enzymatic methylation and cell differentiation.

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