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Targeting "Hydrolytic" Activity of the *S*-Adenosyl-L-Homocysteine Hydrolase

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Abstract: Substrates that are specific for the "hydrolytic" activities of AdoHcy hydrolase have been recently identified. Upon interaction with the AdoHcy hydrolase such substrates generate the "active" electrophiles which then react with the enzyme nucleophiles to produce covalent inhibition. Dihalohomovinyl and haloacetylene analogues derived from adenosine as well 5'-*S*-allenyl-5'-thioadenosine derivative have been characterized as the first type II mechanism-based inhibitors of AdoHcy hydrolase that rely only on the "hydrolytic" activity. Design and synthesis of the novel adenine nucleosides as well their interaction with AdoHcy hydrolase are discussed in this review.

INTRODUCTION

The cellular enzyme *S*-adenosyl-L-homocysteine hydrolase (AdoHcy hydrolase; EC 3.3.1.1) effects hydrolytic cleavage of AdoHcy to give adenosine (Ado) and L-homocysteine (Hcy) [1,2]. AdoHcy is a potent feedback inhibitor of crucial transmethylation enzymes that involves *S*-adenosyl-L-methionine (AdoMet), and alteration of the cellular AdoMet/AdoHcy ratio results in serious perturbation of biological methylation of viral RNA [2]. Therefore, design of inhibitors of AdoHcy hydrolase represents a rational strategy for mechanism-based antiviral chemotherapy [3,4], and correlations between their antiviral [5] (including HIV-1 replication [5b]) and cytostatic potencies and their inhibitory effects have been demonstrated [6]. AdoHcy hydrolase inhibitors were also reported to have immunosuppressive effects in animal models [7].

AdoHcy hydrolase plays a key role in controlling the intracellular level of Hcy that is a cystathionine synthetase-catalyzed precursor to cysteine and methionine. Metabolism of AdoHcy by this enzyme is the only known source of Hcy in mammalian cells [8]. Moreover, elevated plasma Hcy levels in humans have been demonstrated to be a risk factor for coronary artery disease in clinical studies [9]. Although supplementation with B-vitamins and folic acid has been shown to be effective in lowering plasma Hcy level in homocysteinemia patients with residual activity of cystathionine synthetase [4a,9b], inhibitors of AdoHcy hydrolase have also the potential to regulate plasma level of Hcy [4a] and hence ability to reduce risk of developing coronary artery disease [9].

Palmer and Abeles investigated [10] the mechanism of AdoHcy hydrolase and found that the reversible mechanism is initiated by oxidation of the hydroxyl function at C3' of AdoHcy **1** by enzyme-bound NAD^+ to give 3'-keto-nucleosides **2** ("oxidative" activity of the enzyme) (Fig. **1**). This activates H4' for elimination of Hcy to give enone **3** Michael-type addition of water ("hydrolytic" activity) give 3'-ketoAdo **5** which is reduced by enzyme-bound NADH to give Ado **4**. They also discovered that 4',5'-didehydro-5'-deoxyadenosine **6** functioned as an alternative substrate and was oxidized at C3' to give enone **3** directly. Isotope studie with 3'-[^{2}H]-(Ado and AdoHcy) [11] and C5'-deuterium labeled 4',5'-didehydro-5'-deoxyadenosine [12] as well Porte [13] general kinetic studies reinforced the Palmer and Abele mechanism [10].

It has long been assumed that the Palmer and Abele mechanism [10] operated in sequential fashion and tha oxidation of the hydroxyl function at C3' to form the 3' ketone (**1** **2**) ("oxidative" activity) is a prerequisite fo conjugated addition of water ("hydrolytic" activity) across th double bond of the activated enone **3**. However, Borchard and coworkers have shown that those two catalytic activitie of AdoHcy hydrolase can be independent of each other [14] They defined type I mechanism-based inhibitors of AdoHc hydrolase as inhibitors which serve as substrates for th "oxidative" activity of the enzyme. Such inhibitors afford th 3'-keto derivative of the inhibitor and convert the enzym from its active form (NAD^+) to its inactive form (NADH (cofactor depletion mechanism). Type II mechanism-base inhibitors of AdoHcy hydrolase are envisioned to utilize th "oxidative" and/or "hydrolytic" activity of the enzyme to generate electrophiles at the active site, which could the react with enzyme nucleophiles to covalently modify th enzyme (covalent inactivation mechanism) [2,4]. Type II inhibitors are those that utilize neither "oxidative" o "hydrolytic" activity, but reversibly bind to the enzyme [4].

The X-ray crystal structure of a substrate-bound NADH form of human AdoHcy hydrolase has been determined [15] In this pivotal experiment the pure NAD^+–form of th enzyme was inactivated with 9-(2,3-dihydroxycyclopent-4-en-1-yl)adenine [**7**, DHCeA; (Fig. **2**)] to give crystal of th 3'-ketoDHCeA/NADH form of human AdoHcy hydrolas suitable for X-ray crystallographic analysis. The sequestere water molecule at the active site was found to be hydrogen bonded to His55, Asp131 and His301. The water molecul

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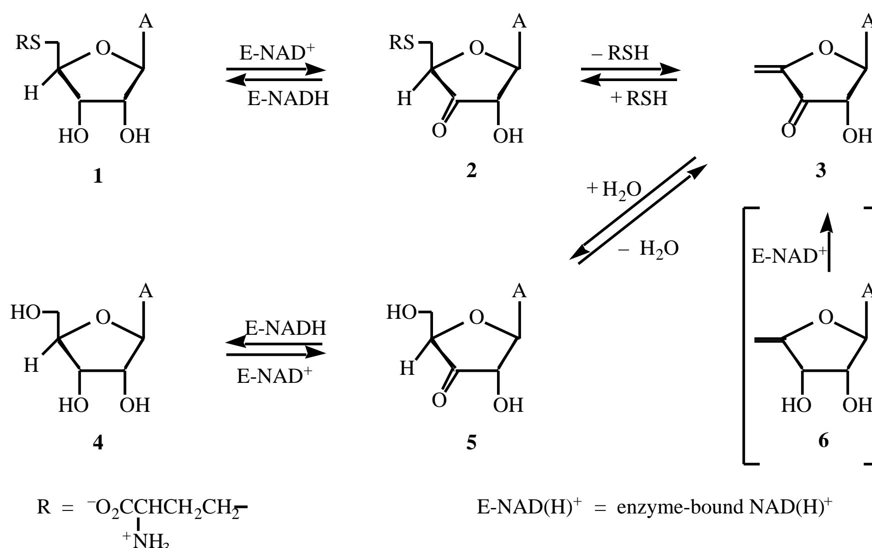

Fig. (1). Proposed mechanism [10] for *S*-adenosyl-L-homocysteine hydrolase.

appears to have a dual role in the catalytic mechanism. It not only is the sole candidate for the catalytic base responsible for the H4' abstraction initiating Hcy elimination (**2** **3**) but also probably adds to the intermediate enone **3** in the formation of 3'-ketoAdo **5** [4b,15]. A recent crystal structure of AdoHcy hydrolase from rat liver in the substrate-free NAD^+ form shows an open catalytic site in the absence of substrate [16]. It identified Glu155 as a proton acceptor from the 3'-OH during abstraction of the H3' by NAD^+ and His54 or Asp130 as general acid–base catalyst. The Cys194 was proposed to modulate the oxidation state of the bound NAD^+. Both crystal structures do not unequivocally define the binding site for the homocysteine moiety of AdoHcy at the active site of the enzyme.

THE 5'-MODIFIED ADENOSINE ANALOGUES

5'-Halo-4',5'-Unsaturated Adenosine Derivatives

McCarthy and coworkers designed and synthesized vinyl fluoride **8** (4',5'-didehydro-5'-deoxy-5'-fluoroAdo) as a potential type II mechanism-based inhibitor which also possessed significant biological activity [17]. Synthesis of **8** have employed fluorination of the suitable protected 5'-*S*-aryl-5'-thioadenosine analogues (e.g. sulfoxides **11**) with (diethylamino)sulfur trifluoride (DAST) to yield the -fluoro thioethers **13** (Fig. **3**). Selective oxidation of **13** with *meta* chloroperoxybenzoic acid (*m*-CPBA) afforded the -fluor sulfoxide diastereomers **14** [17b, 18]. Thermal elimination o sulfenic acid from **14** and deprotection gave the vinyl fluorid **8** and its *E*-isomer. The crucial fluorination step of sulfoxide **11** as well thioethers **12** was improved with DAST/SbCl combination [19] and the relevant chemistry have bee reviewed [20, 21].

Borchardt and coworkers showed [14] that vinyl fluorid **8** is not a type II inhibitor but rather a "pro-inhibitor" that i converted by the AdoHcy hydrolase into adenosine 5' aldehyde **10** (and its 4'-epimer) which inactivates the enzym by the type I mechanism. Release of the fluoride anion fron **8** is effected by the "hydrolytic" activity of this enzyme an was independent of its "oxidative" activity [2b, 14]. Th Ado-5'-aldehyde **10** was independently synthesized an shown to be equally potent inhibitor of AdoHcy hydrolas [22].

Treatment of the protected thioether **12** or it diastereomeric sulfoxides **11** with iodobenzene dichloride o sulfuryl chloride/pyridine gave the -chlorinated derivative that were thermolyzed and deprotected to give 5' chloromethylene analog **9**, its *E*-isomer, and th corresponding 5',5'-dichloro derivative [17b, 23]. Th authentic *Z*-isomer **9** was found to be a time-dependen

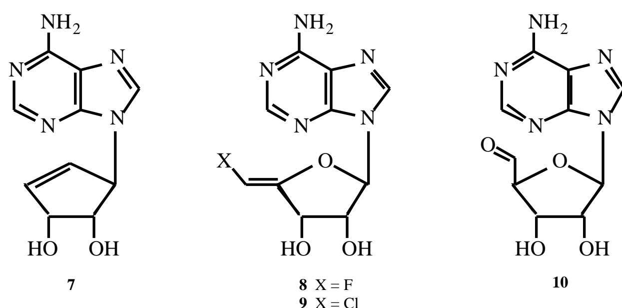

Fig. (2). Selected inhibitors of the AdoHcy hydrolase.

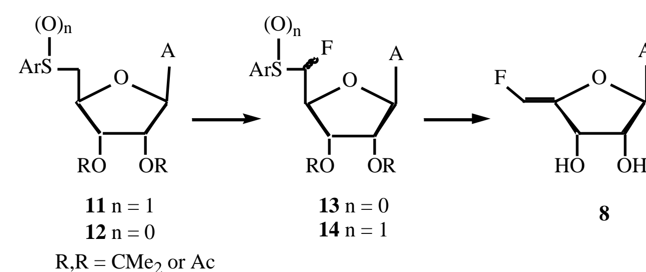

Fig. (3). Synthesis of the vinyl fluoride **8**.

inactivator of the AdoHcy hydrolase with potency comparable to that of its 5'-fluoromethylene analogue **8** [21a, 23].

Miscellaneous 5'-Modified Adenosine Derivatives

Although epimeric Ado-5'-aldehydes **10** are potent inhibitors of AdoHcy hydrolase, they are also somewhat unstable and difficult to purify and characterize especially when deprotected [22]. Such aldehydes form hydrates and undergo isomerization at C4' presumably via the hydroxy enol ether **15** (Fig. **4**). Therefore, we considered that the oxime derivatives **17** might serve as AdoHcy hydrolase-activated prodrugs that would undergo hydrolysis to yield 5'-aldehydes **10** within the enzyme active site [24]. Also, the 5'-*O*-methyl hydroxy enol ether **16** (stable vinyl diether) was targeted [18b].

The crystalline Ado-5'-aldehyde oximes and their arabino, 2'-deoxy, and 3'-deoxy analogues have been prepared [24a]. Oximes **17** (*E/Z*, 4.5:1) were obtained from 6-*N*-benzoyl-2',3'-*O*-isopropylideneadenosine in four steps [Moffatt or Dess-Martin oxidation to give the suitable protected 5'-aldehyde followed by treatment with the hydroxylamine hydrochloride and successive removal of benzoyl (NH_3/MeOH) and isopropylidene (trifluoroacetic acid/H_2O) protection groups] in 67% yield without isolation and purification of the intermediates. Oximes **17** are potent inhibitors of AdoHcy hydrolase and are cytotoxic against several tumor cell lines [24a]. The active species generated from **17** by the "hydrolytic" activity of the enzyme were determined to be Ado-5'-aldehydes **10** which are then oxidized in a slower step to the 3'-ketoAdo-5'-aldehydes producing type I inhibition [24b]. The vinyl diether **16** was stable in buffer solution used for the enzyme assays but functioned as a moderately potent time-dependent inactivator of the AdoHcy hydrolase [18b]. Analogously to oximes **17**, compound **16** is converted to the Ado-5'-aldehydes **10** in the active site of the enzyme provides further support for the ability of AdoHcy hydrolase to hydrate and/or oxidized enol intermediates [24b].

The sugar-modified adenosine 5'-aldehydes and their oxime derivatives were also prepared. It was found that removal of the 2'-hydroxyl group (e.g. 2'-deoxyAdo oximes **18**), or 2'-epimerization (e.g. AraAdo oxime derivatives **19**) resulted in reduced activity, whereas removal of the 3'-hydroxyl group (e.g. 3'-deoxyAdo oximes **20**) abolished activity [24a]. These trends are in harmony with a mode of action of AdoHcy hydrolase involving binding in the enzyme active site with the consensus that ribo *cis*-glycol unit is

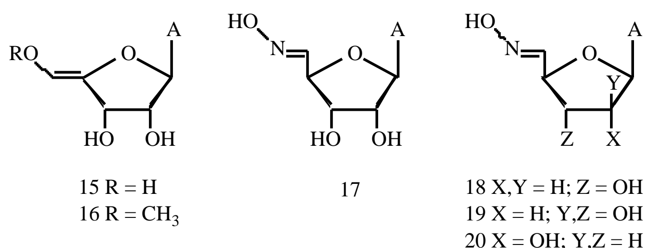

Fig. (4). AdoHcy hydrolase-activated "pro-inhibitors" of Ado-5'-aldehydes

required for good binding. It appears that the 3'-hydroxy group is essential for adequate binding with AdoHc hydrolase, and such binding apparently is prerequisite for th hydrolytic action of the enzyme as well as for the mandator oxidation to produce type I inhibition (*vide infra*).

To further investigate the 5'-hydrolytic capability c AdoHcy hydrolase, ester **21** and amide **22** derivatives of th adenosine 5'-carboxylic acid **23** (Fig. **5**) were prepared [25] Treatment of **23** with thionyl chloride and the correspondin alcohols gave esters **21**. Ado-5'-carboxamide and *N* substituted amides **22** were prepared from **23** by ammono lysis or dicyclohexylcarbodiimide-mediated coupling wit the corresponding primary and secondary amines. In contras to vinyl fluoride **8** and oximes **17**, no involvement of th

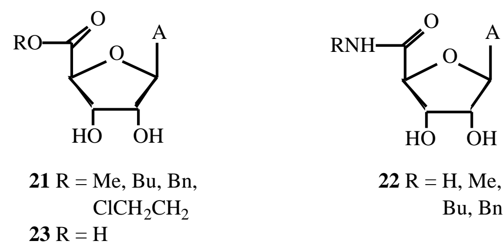

Fig. (5). Adenosine 5'-carboxylic acid derivatives.

"hydrolytic" activity of the AdoHcy hydrolase durin inhibition by esters **21** or amides **22** was detecte Observation of the reduction of NAD^+ to NADH indicate that they are cofactor depletion type I inhibitors [25]. It i noteworthy that Ado-5'-carboxylic (uronic) acid derivative are selective adenosine receptor agonists with cardiovascula properties [26].

THE HOMOADENOSINE ANALOGUES

6'-Halo-5',6'-Unsaturated Adenosine Derivatives

We were fortunate to find [6, 27] that AdoHcy hydrolas is also capable of adding water, at either terminus, across th isolated 5',6'-double bond of the 6'-halo(vinyl) homoAd derivatives **24** (Fig. **6**). A sulfone-stabilized Wittig reagen with subsequent vinyl-sulfone and organotin chemistry wer employed for the synthesis of homovinyl halides **24** [6] Thus, Moffatt oxidation of 2',3'-*O*-isopropylideneadenosin and treatment of the crude 5'-aldehyde **25** with [(*p* tolylsulfonyl)methylene]triphenylphosphorane gave the 6' tosyl(vinyl) sulfone **26** as *E* isomer in high yields [28] Radical stannyldesulfonylation (Bu_3SnH/AIBN/toluene/) o **26** gave separable mixtures of the vinyl 6'-stannanes **27** (*E/Z* >6:1) (Fig. **7**). Quantitative and stereospecific halodestanny lation of **27** (*E*, or *Z*-isomer) occurred with I_2 and Br_2 (or th respective *N*-halosuccinimides) to provide the Wittig-type 6' iodo(and bromo)vinyl homoAdo **24** (*E*, or *Z*-isomers) afte deprotection and purification. Treatment of **27** wit XeF_2/silver triflate [29] effected fluorodestannylation to giv **24** (X = F) and chlorine converted **27** to 6'-chloro derivative **24** (X = Cl, *E* and *Z*) but with lower stereoselectivity [6].

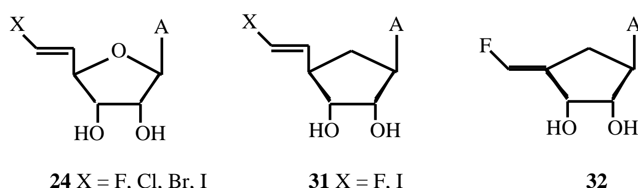

Fig. (6). The 6'-Halo(vinyl)homoadenosine and related aristeromacin analogues.

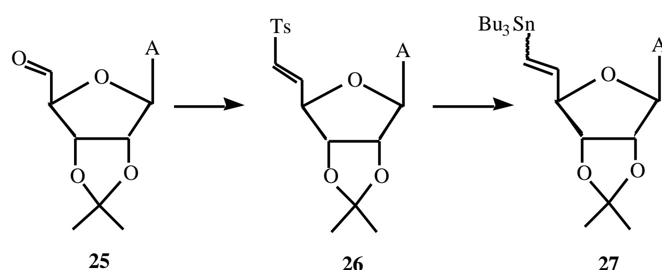

Fig. (7). Synthesis the 6'-halo(vinyl) homoadenosine analogues.

The 6'-halo(vinyl) homoAdo analogues **24** are concentration- and time-dependent inactivators of AdoHcy hydrolase. The inhibition potencies were correlated with anticancer and antiviral activities of **24** and found to be in the order of I > Br > Cl > F (and *E* > *Z*) [6]. Amazingly, AdoHcy hydrolase possesses the catalytic power to effect addition of water to the isolated double bonds of **24** [27]. AdoHcy hydrolase effected hydrolysis of the 6'-halogen from **24** via the addition of H_2O at C6' followed by the halide elimination to produce homoadenosine 6'-aldehyde **28** which undergoes spontaneous decomposition to Ade (Fig. **8**). Addition of H_2O at C5' of **24** (X = F) results in the formation of 6'-deoxy-6'-fluoro-5'-hydroxyhomoadenosine **29**. In a third pathway, enzymatic oxidation of **24** provides 3'-ketone **30** which could react with water at either the C5' or C6' position

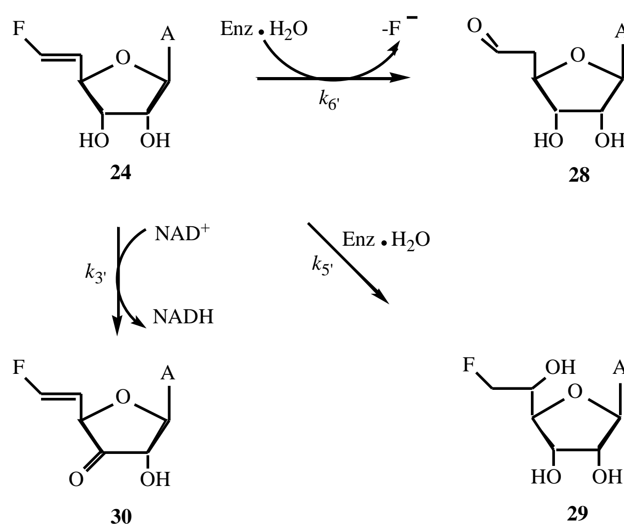

Fig. (8). Inactivation of AdoHcy hydrolase by the 6'-fluoro(vinyl) homoadenosine

[27]. The hydrolytic (C5'/C6') and oxidative (C3') activitie of AdoHcy hydrolase are differentiated most effectively witl the 6'-fluoro analogue **24** (X = F) with partition ratio amon three pathways were found to be $k_{3'}$:$k_{5'}$:$k_{6'}$ = 1:79:29 [27b] Lys-426 was identified as a crucial residue for the enzymati hydrolysis of **24** (X = F) [30].

Aristeromycin Analogues

To eliminate ribosyl ring cleavage during inhibition o AdoHcy hydrolase by **24** the 6'-halo(vinyl homoaristeromycin derivatives **31** (see Fig. **6**) were prepare in which the furanosyl ring oxygen was replaced by methylene unit [31]. Such modification was expected t provide analogues that could not suffer cyclopentanyl rin cleavage by -elimination (H5' and the ring oxygen O4') a observed for homoadenosine 6'-aldehyde **28**. The haloviny aristeromycin analogues **31** were prepared employing simila vinyl-sulfone and organotin chemistry as showed in (Fig. **7** for adenosine derivatives. Inactivation of AdoHcy hydrolas by **31** (X = F) involved addition of water at the vinyl C5' o C6' (with elimination of fluoride) and oxidation at C3' [a showed in (Fig. **8**) for adenosine analogues]. The partitio ratio among three pathways were determined to be $k_{3'}$:$k_{5'}$:$k_{6'}$ = 1:0.6:1.7.

It is noteworthy that the 4',5'-didehydro-5'-deoxy-5' fluoroaristeromycin **32** was not a substrate for the hydrolyti activity of the enzyme since incubation of AdoHcy hydrolas with **32** did not result in the release of fluoride ion [32] However, both **32** and independently synthesize aristeromycin 5'-aldehyde were potent type I inhibitor [32,33]. It is possible that enzyme-mediated protonation o the ribosyl ring oxygen of **8** (as well **24**) enhances th electrophilicity of the C5', making the 5' position mor susceptible to attack by the enzyme-bound water.

3'-Modified Vinyl Halides

In order to probe "pure" hydrolytic activity of th AdoHcy hydrolase, analogues of vinyl halides **8** and **2** without an oxidizable function (hydroxyl group) at C3' hav been targeted [34]. The 3'-deoxy modification gave analogue (e.g. **33** and **36**) with greater differences in stereoelectroni effects and lack of a hydrogen-bond acceptor at C3' (Fig. **9**) In other series, the 3'-hydroxyl group has been replaced witl fluoro **34** or chloro **35** group to give closer similarities witl the natural hydroxy substituent, but still preclude oxidativ

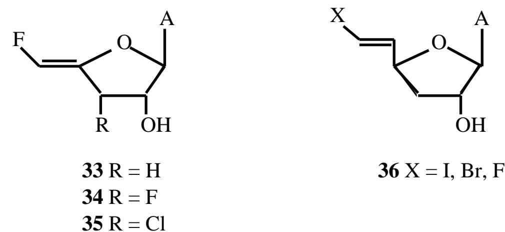

Fig. (9). The 3'-modified vinyl halides analogues of Ado an homoAdo

substrate activity at C3'. The 3'-modified analogues **33-36** were weak inhibitors of AdoHcy hydrolase and in sharp contrast with the 3'-hydroxy analogues **8** and **24** were not substrates for the "hydrolytic" activity of the enzyme (no formation of halide ions, 5'-aldehyde analogues and adenine was detected in enzymatic assays) [34]. Therefore it is safe to conclude, that the 3'-hydroxyl group is essential for effective substrate binding to AdoHcy hydrolase, and such binding must be required for execution of the "hydrolytic" activity of the enzyme (*vide supra* results with 3'-modified Ado-5'-oximes **20**).

Dihalovinyl Homoadenosine Analogues

The geminal and vicinal (dihalo)homovinyl analogues **37-39** were also designed as putative new substrates for the "hydrolytic" activity of AdoHcy hydrolase (Fig. **10**). Treatment of the crude Ado-5'-aldehyde **25** with (bromofluoromethylene)triphenylphosphorane (generated in situ with $CBr_3F/Ph_3P/Zn$) and deprotection gave the

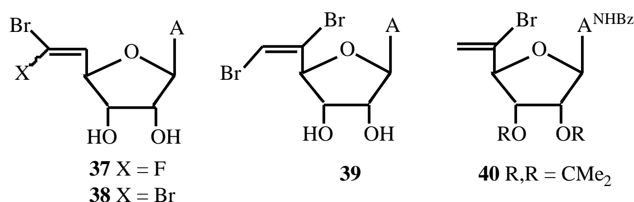

Fig. (10). The dihalovinylhomoadenosine analogues.

bromofluorovinyl diastereomers **37** (*E/Z*, ~3:2) [35]. Analogous treatment of **25** with dibromomethylene-Wittig reagent provided the geminal dibromo derivative **38**. Bromination of the 5'-bromohomovinyl derivative **40** (to give the 5',5',6'-tribromo compound) followed by amine-mediated dehydrobromination and deprotections afforded the vicinal 5',6'-dibromohomovinyl derivative **39** as a single diastereomer [35].

The [bromo(fluoro)]homovinyl analogue **37** covalently modifies human placental AdoHcy hydrolase and was found to be the first type II inhibitor which utilizes only the "hydrolytic" activity [36]. Thus, enzyme-mediated addition of water to **37** at C6' of the 5',6'-double bond followed by elimination of bromide ion generates an electrophilic acyl fluoride **41** (Fig. **11**). The acyl halide then undergoes nucleophilic attack by a proximal Arg196-NH_2 group to form a covalent adduct **42** (lethal event). The enzyme retains its original NAD^+/NADH content indicating no oxidation at C3' with a maximum inactivation of 83%. In a second nonletha event depurination and hydrolysis of **41** (with elimination o fluoride ion) produced hexose-derived 6-carboxylic acid [36].

Acetylene and Haloacetylene Analogues

Parry and coworkers have shown that an acetyleni analogue of adenosine **43** is a type II inhibitor of AdoHc hydrolase [37]. This acetylenic analogue deactivates th enzyme first by serving as a substrate for the 3'-oxidativ activity (converting NAD^+ to NADH) and producing the 3' keto acetylene **44** (Fig. **12**). It is proposed that this tightl bound intermediate isomerizes to an electrophilic alleni ketone **45** which then reacts with an active protei nucleophile to give adduct **46**. The putative covalent linkag was found to be acid sensitive but stable to mild base Histidine, arginine, cysteine or lysine residues were hinted a possible nucleophiles [37]. The 4'-acetylenic analogue **4** possesses also antiviral and cytostatic activities [6, 38].

In a search for more specific inhibitors that exploi "hydrolytic" activity of the AdoHcy hydrolase, 6'-haloace tylenic adenine nucleosides **47** (X = Cl, Br, I) (Fig. **13**) wer designed by Robins and coworkers [39]. Thus, treatment o dibromomethylene **38** with excess BuLi effecte dehydrobromination to provide the acetylenic derivative **4** in a moderate yield [38,39]. Treatment of 2',3'-*O* isopropylidene-**43** with *N*-iodosuccinimide (NIS)/AgNO resulted in efficient 6'-iodination to give **47** (X = I) afte deprotection. The bromo **47** (X = Br), and chloro **47** (X = Cl) acetylene analogues were prepared in a higher overal yield from the corresponding acetylene sugars which wer coupled with adenine [39].

Surprisingly the haloacetylenic nucleosides **47** inactivat the AdoHcy hydrolase by different mechanisms. The 6' bromo- and 6'-chloroacetylenic **47** (X = Cl, Br) produce partial (~50%) loss of enzyme activity with a concomitan (~50%) reduction of NAD^+ to NADH. In addition, Ade an halide ions were released and the enzyme was found to b covalently bound to Lys318 [40]. Addition of enzyme sequestered water at the 5' position followed b tautomerization of the hydroxyvinyl intermediates i suggested to generate -halomethyl ketones **48** at th enzyme active site. Attack by a proximal nucleophil (Lys318) forms enzyme-inhibitor covalent linkage **49** whicl is oxidized slowly to a corresponding 3'-keto adduct. In parallel pathway addition of water at 6' position produces a acyl halides **50** (X = Br, or Cl) which chemically degrad into Ade, halide ion, and sugar-derived products. Covalen

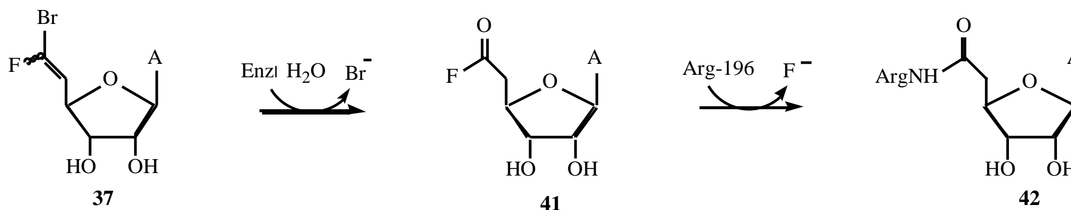

Fig. (11). Possible mechanism by which 6'-bromo-6'-fluorohomovinyladenosine **37** inactivates AdoHcy hydrolase.

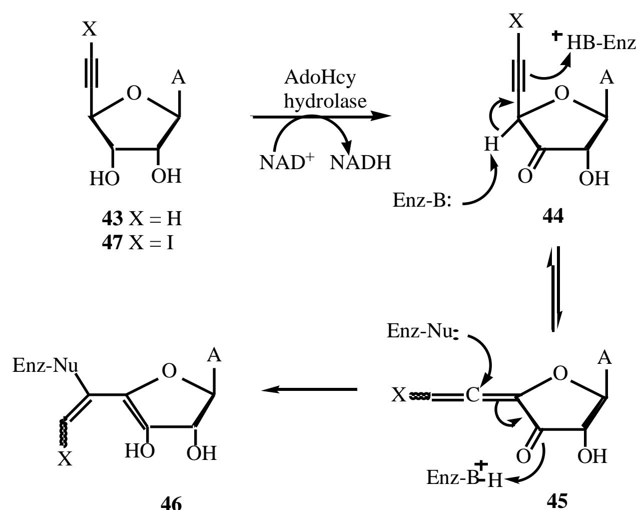

Fig. (12). Mechanism of inactivation of AdoHcy hydrolase by the acetylene derivative **43** and its 6'-iodo analogue **47**.

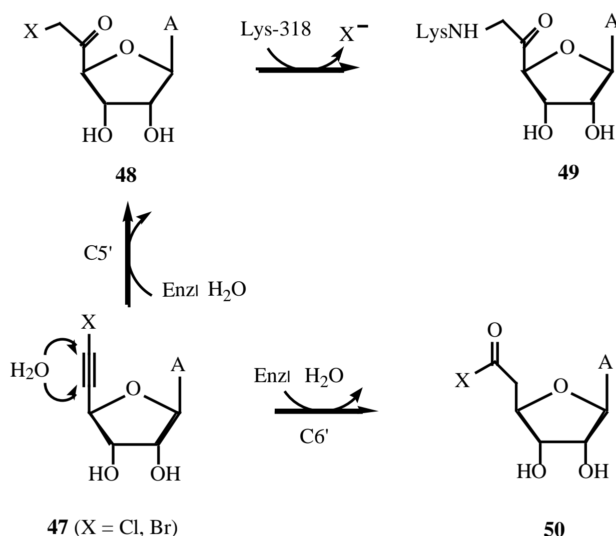

Fig. (13). Mechanism of inactivation of AdoHcy hydrolase by 6'-chloro(or bromo) acetylene analogues **47**.

modification of **50** were not detected probably due to its instability and much lower partition ratio of the 6' to 5' hydrolytic activity (~1:60) [40].

In contrast to 6'-bromo(or chloro)acetylenes **47** (X = Br, Cl), the 6'-iodo compound **47** (X = I) completely inactivates the enzyme with experimental observations [40] consistent with the results reported by Parry and coworkers [37] for the unsubstituted acetylenic anlogue **43** depicted in (Fig. **12**). The data suggest involvement of the 3'-oxidative activity in the "activation" of the inhibitor **47** (X = I), resulting in a type II mechanism of inactivation. However, because of the instability of the inhibitor-enzyme complex **46** (X = H or I), the specific amino acid residues that are covalently modified by these inhibitors were not identified [37,40]. Nevertheless, crystal structure of an AdoHcy hydrolase in substrate-bound form shows three nucleophilic amino acids residues (His55 His301 and Asp131) close to the 5'-position of the substrat [15].

It is noteworthy that dihalohomovinyl inhibitor **37** wa primary converted by the 6'-hydrolytic activity of the enzym to the acyl fluoride **41** which was further covalently modifie by Arg196. On the other hand, the haloacetylenic inhibitor **47** (X = Br, Cl) were mainly transformed by the 5' hydrolytic activity of the enzyme to the -halomethy ketones **48** which were covalently modified by Lys318 These different pathways reflect the differences in the chemica reactivity and/or juxtaposition of the -halomethyl ketone **48** versus the acyl fluoride **41** with the nucleophiles near th enzyme's active site.

"Doubly Homologated" Adenosine Derivatives

The "doubly homologated" vinyl halides **51** an acetylenic **52** adenine nucleosides (Fig. **14**) were designe because these derivatives (sp^3 hybridized C5') should hav greater conformational flexibility at C5' relative to analogue that have vinylogous (sp^2; **24** or **37**) or acetylenic (*sp*; **43** o **47**) functions directly attached to the ribose ring [41]. The also provide probes for evaluation of tolerated distances i the enzyme between the binding site for O3' (involved wit oxidation at C3') and the protein residues which ar responsible for the "hydrolytic" activity (already shown t function at C5' and C6').

Synthesis of the "doubly homologated" 6',7'-unsaturate analogues **51** and **52** began with *ribo*-hexofuranose suga precursor of type **54** [41], because homologation of adenosin to homoadenosine required multi-step procedures which gav low overall yields and homoAdo 6'-aldehyde is known [27a to be very unstable (Fig. **15**). Thus, 1,2-*O*-isopropylidene-D-glucose **53** was converted to the protected -D-*ribo* hexofuranose **54** in good overall yield with key step involved selective deoxygenation at C5 and inversion c

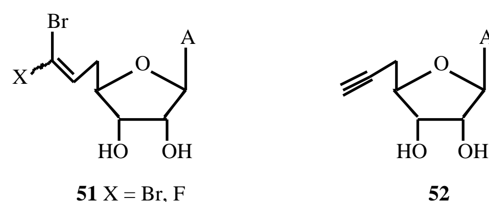

Fig. (14). The doubly homologated dihalovinyl and acetylen analogues of adenosine.

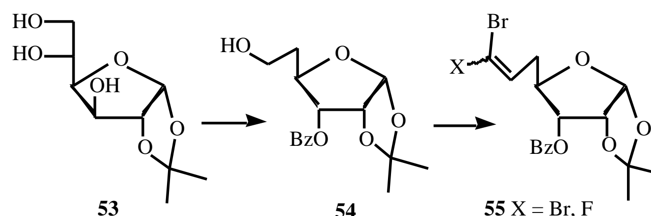

Fig. (15). Synthesis of homologated dihalovinyl sugar pre-cursors from glucose.

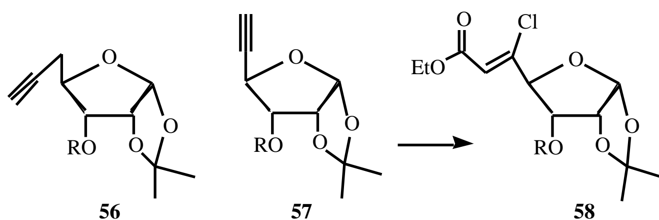

Fig. (16). Synthesis of doubly functionalized vinylic ribofuranoses.

configuration at C3. Wittig-type olefination of **54** with (dihalomethylene)triphenylphosphorane [CBr_4(or CBr_3F)/ Ph_3P/Zn] gave dihalovinyl-heptofuranoses **55** (X = F or Br) [41]. The anomeric acetates derived from **55** were coupled with adenine and deprotected to give dihalovinyl nucleosides **51** (49%). Treatment of **51** (X = Br) with excess BuLi effected dehydrobromination to give the acetylenic derivative **52** [41].

A one-step conversion of ribofuranose acetylenes **56** and **57** (Fig. **16**) into vinylic derivatives functionalized with chloro and ethoxycarbonyl groups (e.g. **58**) as precursors for the synthesis of the corresponding adenine nucleosides which may interact with AdoHcy hydrolase has been reported [42]. Thus, treatment of the ribose-derived terminal alkyne **57** with ethyl chloroformate in the presence of a catalytic amount of $RhCl(CO)(PPh_3)_2$ in toluene [43] effected *syn* chloroalkoxycarbonylation to give doubly functionalized vinylic derivative **58**.

5'-THIOADENOSINE DERIVATIVES

Covalent inhibition of AdoHcy hydrolase with 5'-deoxy-5'-difluoromethylthioadenosine **59** (Fig. **17**) by liberation of the electrophilic entity from the substrate has been reported by Guillerm and coworkers [44]. The proposed inactivation of **59** involves enzymatic oxidation and -elimination of the difluoromethylthiolate ion from the 3'-keto intermediate to give the enone **3** and thioformyl fluoride **61** (from difluoromethylthiolate). Generated within the enzyme cavity highly electrophilic **61** was proposed to irreversibly acylate nucleophilic residues of the AdoHcy hydrolase. Interestingly the 2'-deoxy and 3'-deoxy analogues (e.g. **60**) also caused irreversible inactivation of the enzyme [45]. Inactivation of **60** was accompanied by release of fluoride ion with retention of NAD^+/NADH content. Two hypothetical mechanisms were suggested. One involved generation of the same thioformyl fluoride **61** (without C3' oxidation). In a second mode of inactivation, formation of 5'-thio intermediate **64** generated by the hydrolytic activity of the enzyme (addition of water to **62** to give **63**) was considered. Formation of a disulfide bond with a thiol intermediate **64** and a cysteine residue was proposed to explain the covalent inactivation [45].

Guillerm and coworkers have also developed [46] new series of 5'-thioadenosine analogues substituted at sulfur with allenyl **65** and propynyl **70** groups (Fig. **18**). Compounds **65** and **70** caused potent time-dependent and irreversible inactivation of the enzyme. The inactivation was concomitant with the formation of Ado (or AdoHcy in the presence of Hcy) but the NAD^+/NADH content was not affected. The proposed preliminary mechanism involves addition of enzyme sequestered water to the *S*-allenyl group of **65** to generate reactive thioester **66**. Attack by amino functionalities might cause type II covalent inhibition (e.g. **67**). A process involving -elimination without prior oxidation at C3' yielding and allenyl mercaptan, whose tautomeric thioaldehyde **68** is highly reactive acylating agent to produce covalent adduct of type **69** was also considered [46].

MISCELLANEOUS ADENOSINE DERIVATIVES

Among other mechanism-based inhibitors of AdoHcy hydrolase, 4'-methyl substituted AdoHcy **71** [47], 4'-fluoroadenosine **72** [48] and 4'-fluoromethyladenosine **73** [49

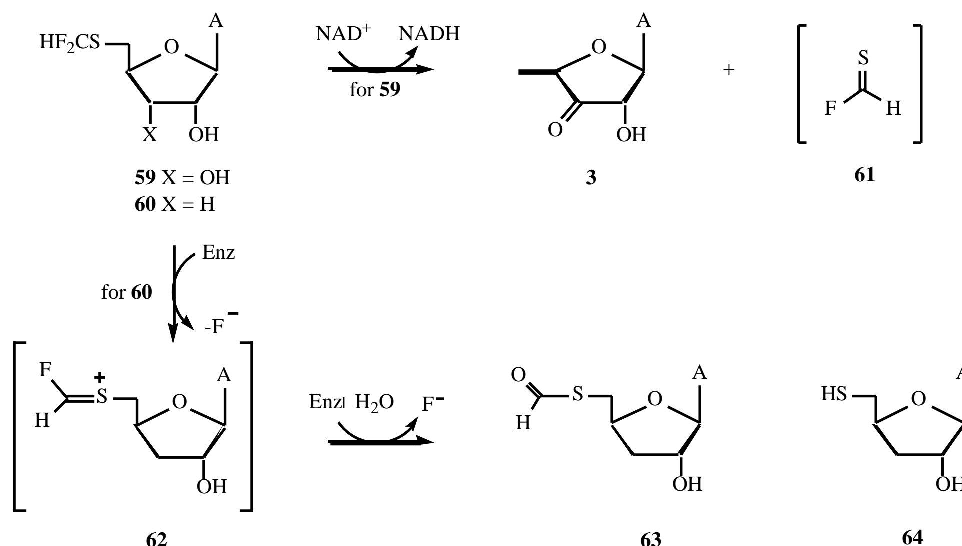

Fig. (17). Proposed mechanism for the conversion of 5'-*S*-difluoromethyl-5'-thioadenosine analogues by AdoHcy hydrolase

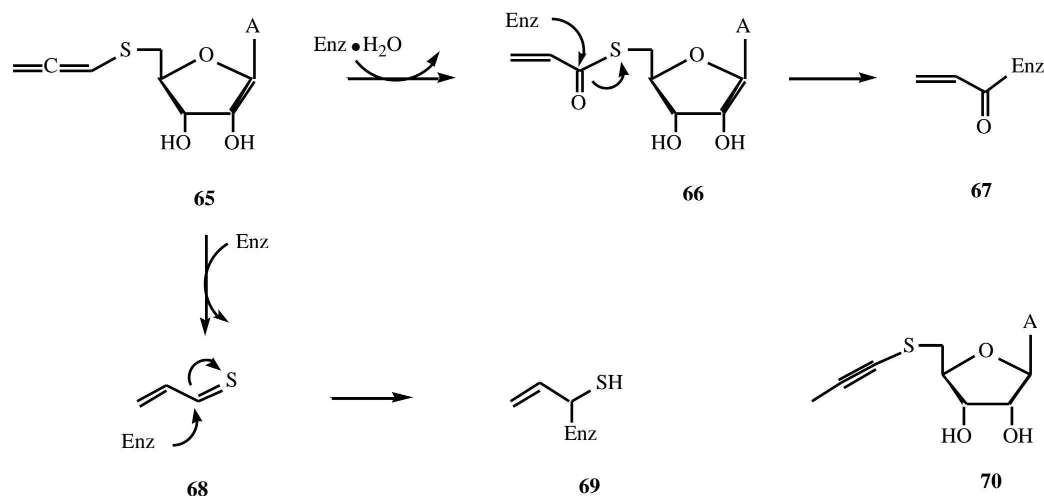

Fig. (18). Inactivation of AdoHcy hydrolase by 5'-*S*-allenyl-5'-thioadenosine.

have been synthesized (Fig. **19**). Lacking H4', such compounds can not participate in the elimination step of the Palmer and Abeles mechanism [10] and may function directly or indirectly as inhibitors of AdoHcy hydrolase. The 4'-fluoroadenosine **72** caused a time-dependent inactivation of the enzyme which was accompanied by reduction of the enzyme-bound NAD^+ to NADH [48].

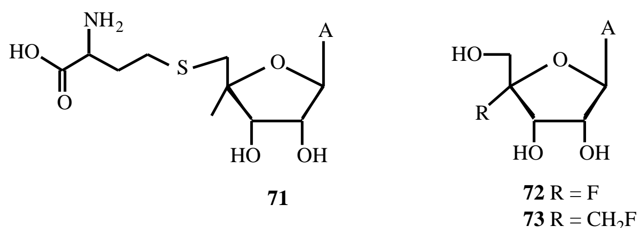

Fig. (19). The C4'-substituted adenosine analogues.

Parry and coworkers investigated [50] the inhibition of AdoHcy hydrolase by 2',2'-difluoroadenosine **74** which is known to exhibit potent cytotoxity toward human leukemia cell lines (Fig. **20**). The presence of the 2',2'-difluoro functionality was expected to increase both stability of the putative 3'-keto intermediate **75** towards glycosidic cleavage and its electrophilicity. Formation of a Schiff base linkage with the putative lysine residue resulting in the enzyme-inhibitor complex (e.g. **76**) was expected. However, all attempts to stabilize the complex **76** failed.

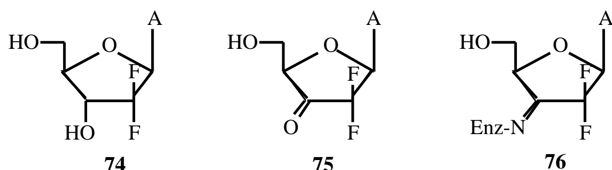

Fig. (20). The 2'-deoxy-2',2'-difluoroadenosine and hypothetical inactivation products with AdoHcy hydrolase.

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ABBREVIATIONS

AdoHcy hydrolase	=	*S*-Adenosyl-L-homocysteine hydrolase
AdoHcy	=	*S*-Adenosyl-L-homocysteine
AdoMet	=	*S*-Adenosyl-L-methionine
Ade	=	Adenine
Ado	=	Adenosine
Bz	=	Benzoyl
m-CPBA	=	*meta*-Chloroperoxybenzoic acid
DAST	=	(Diethylamino)sulfur trifluoride
DHCeA	=	9-(2,3-Dihydroxycyclopent-4-en-1-yl)adenine
Hcy	=	L-Homocysteine
HIV	=	Human Immunodeficiency Viruses
NAD^+/ NADH	=	Nicotinamide adenine dinucleotide
NIS	=	*N*-Iodosuccinimide
Ts	=	*p*-Toluenesulfonyl

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